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Title of Thesis: "Identification and Quantification of Pesticides in Environmental Waters with Solid Phase Microextraction and Analysis using Field-Portable Gas Chromatography-Mass Spectrometry

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A solid-phase microextraction (SPME) and gas-chromatography-mass spectrometry (CG-MS) sampling and analysis method was developed for 1-Naphthyl methylcarbamate (Carbaryl) and gamma-benzenehexachloride (gamma-BHC) in water. A 15 minute sampling time of 40 degrees centigrade with a carbowax/divinylbenzene-coated SPME fiber for carbaryl and a polydimethylsiloxane-coated SPME fiber for lindane was employed. This allowed detection of carbaryl at concentrations in environmental water sources that included modeled ground water, simulated post-production water, and raw surface water while using the MS detector in full scan mode. The method was also successfully used with a field-portable CG-MS instrument using a low thermal mass, resistively heated column (LTM/RHC). Total analysis time using the field-portable CG-MS system was 30 minutes. The method avoids the use of complex sample preparation steps and thereby enhances analyst safety through the elimination of the need to handle solvents in the field environment.

15. SUBJECT TERMS

Pesticides; carbamate; carbaryl; organochlorine; lindane (gamma-BHC); environmental waters; solid phase microextraction (SPME); gas-chromatography-mass spectrometry (CG-MS); field environment; chemical identification methods

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ABSTRACT

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Author: CPT Michael J. Nack
Master of Science in Public Health

Thesis Directed by: CDR Gary L. Hook
Assistant Professor
Department of Preventive Medicine and Biometrics

A solid-phase microextraction (SPME) and gas-chromatography-mass spectrometry (GC-MS) sampling and analysis method was developed for 1-Naphthyl methylcarbamate (Carbaryl) and gamma-benzenehexachloride (gamma-BHC) in water. A 15 minute sampling time at 40ºC with a carbowax/divinylbenzene-coated SPME fiber for carbaryl and a polydimethylsiloxane-coated SPME fiber for lindane was employed. This allowed detection of carbaryl at concentrations as low as 10 µg/L and lindane at 1.0 µg/L in environmental water sources that included modeled ground water, simulated post-production water, and raw surface

water while using the MS detector in full scan mode. The method was also successfully used with a field-portable GC-MS instrument using a low thermal mass, resistively heated column (LTM/RHC). Total analysis time using the field-portable GC-MS system was 30 minutes. The method avoids the use of complex sample preparation steps and thereby enhances analyst safety through the elimination of the need to handle solvents in the field environment.

IDENTIFICATION AND QUANTIFICATION OF PESTICIDES IN ENVIRONMENTAL WATERS WITH SOLID PHASE MICROEXTRACTION AND ANALYSIS USING FIELD-PORTABLE GAS CHROMATOGRAPHY-MASS SPECTROMETRY

BY

CPT MICHAEL J. NACK

Thesis submitted to the faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirement for the Degree of Master of Science in Public Health, 2004

DEDICATION

I want to thank my wonderful wife Christina, and three amazing children Chelsey, Rachel, and Matthew for their support, patience, and understanding during the last two years. The reasons were not always apparent to you for the long hours spent in the office trying to not settle for mediocrity, but I'm sure the results will benefit the whole family in the future. The strength of this military family again prevailed and will so again in the future as long separations are eminent with the current tempo of operational deployments. It would be very easy to give it all up most days so that we could enjoy some semblance of stability, but your understanding, support, and encouragement make it possible to continue. Thank-you all for your sacrifices and I hope you know I love you all so much.

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I'd like to give a special thanks to Dr. Tom Gargan of the U.S. Army Center for Environmental Health Research for providing the base concept and funding for this project. I'm very grateful for your patience and understanding during the long periods of communication silence while I worked towards completion of the project. This was important as it allowed me to develop my personal skills needed to complete this type of research and use some creativity in developing the scope of the project to achieve a product that will hopefully benefit both military and civilian public health entities. I hope that our two organizations will continue the favorable and constructive partnership that we've developed and shared over that last two years.

I'd like to thank each member of my committee for agreeing to guide and mentor me throughout my time here at USUHS. I appreciated your patience, understanding, and encouragement, which made this stressful time more manageable. I hope that I'll have the honor and privilege to work with, or for, each and every one of you again in the future.

I'd like to give a final thanks to LT Doug Parrish for acting as a proxy research advisor/trouble-shooter when CDR Hook was teaching or traveling. You displayed such a high level of patience even after asking you the same things four or five different times and for making the equipment continue to work until I could finally do it myself. Your kindness and generosity were definitely appreciated.

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CHAPTER ONE: INTRODUCTION

Statement of the Problem

Pesticides play an important role in agricultural and non-agricultural operations throughout the world. These compounds are easy to obtain and are routinely used to bolster crop yields. From a public health standpoint, pesticides can be used to effectively reduce the number of disease vectors, which ultimately reduces the incidence of vector-borne diseases. Despite the production of less persistent and natural pesticides, their routine use has resulted in the pollution of ground and surface water sources. A combination of their persistence, ability to bioaccumulate, and toxicity has resulted in varying social health concerns regarding their presence in water sources and supplies.

Since water is such a vital commodity of human existence, regulatory agencies in the United States have established strict standards for pesticide production and use to effectively control and reduce contamination levels to safeguard these resources. The Armed Forces Pest Management Board (AFPMB), on behalf of the Department of Defense (DoD), was established to help regulate pesticide use at military installations and by deployed forces to ensure compliance with federal regulations regarding the use of pesticides. The AFPMB's mission is to ensure that environmentally sound and effective programs are present to prevent pests and disease vectors from adversely affecting DoD operations inside and outside of the continental United States in accordance with DoD Instruction 4150.7, "DoD Pest Management Program, April 22, 1996."[1]

Contamination of water resources still occurs despite the efforts of the United States to reduce pesticide use. As a result, public health agencies at all organizational levels are required to conduct periodic monitoring of wastewaters, drinking water sources, and post-production supplies to monitor contaminate levels and implement appropriate control measures as needed.

Many under developed countries have agricultural based economies and depend more heavily on pesticides to ensure crop yields are sufficient to sustain their populations. Therefore, they have not adopted strict guidelines to control and monitor pesticide use resulting in widespread contamination of their local resources. The extensive level of contamination that can occur in these environmental waters poses a threat to the health of deployed military forces that operate in and around these waters and use them to produce drinking water supplies. Accurate initial and periodic health and safety assessments of water resources are needed to identify contaminated waters that are used for other human activities, and ensure enforcement standards are met. Accurate, sensitive, and rapid analytical methods and equipment are needed that can be used in a field environment to quickly assess pesticide contamination levels in water so health planners can make quick and informed decisions necessary to safeguard human health.

Military and civilian field analytical equipment currently used for water analysis is very limited in regards to its ability to detect and quantify toxic industrial chemicals. The DREL field- water analysis sets produced by the Hach Company of Loveland, Colorado (USA) are commonly used in both the military and civilian sectors. Analytical equipment using photometric and colorimetric detectors are common to these sets, which have limited chemical detection abilities and are not capable of identifying unknown compounds[2]. Due to the equipments inability to detect and quantify a broad range of chemical contaminants, water samples must be sent to certified environmental laboratories for analysis that involves specific, complex, and time consuming methods. For military operations outside of the United States, water samples must be sent out of theaters of operation to environmental laboratories in Europe or the United States where general water quality analysis is completed that includes analysis for a limited number of pesticides and herbicides. Health and safety decision makers are not able to

make quick judgments of water conditions since the analysis results are usually not returned to end-users for several weeks. Therefore, it is important that fast and accurate analytical methods and equipment be available to verify the presence of pollutants in environmental waters. Ideally, equipment and techniques should be employed at the point of use to detect the presence of pesticides and other unknown chemicals that are potentially hazardous to human health. Since pesticides are usually present in water at trace levels, and are often mixed with other contaminants present at higher concentrations, it is important that the method includes an extraction and concentration step to maximize detection capability.

Background

The U.S. Army Center for Environmental Health Research (USACEHR) at Fort Detrick, MD, under the auspice of the U.S. Army Medical Material Command, has established Science and Technical Objective (STO) IV.ME.1999.01. The purpose of this STO is to develop better methods for rapid detection of chemical and microbial contaminants in drinking water that will validate safety for human consumption. The development of new technologies for this purpose would help ensure timely evaluation of water safety to protect deployed forces from incidental or purposeful contamination of this vital commodity with industrial chemicals. Current field water sampling equipment and methods used by deployed forces provide rapid quantitative results for only a very small number of toxic chemicals. The STO was developed to help identify equipment and methods that would achieve sufficient sensitivity in less time, measure a broad spectrum of potential contaminants, and minimize the logistical tail. One critical outcome of the STO is to be able to identify at least one class of pesticide at parts-per-million (ppm) levels within 30 minutes. USACEHR has identified two pesticides of interest (carbaryl and lindane)

from the United States Army Center for Health Promotion and Preventive Medicine (USACHPPM) Technical Guide (TG) 230, Chemical Exposure Guidelines for Deployed Military Personnel as candidates for this STO[3]. TG-230 was developed as a mandate from Department of Defense (DoD) Instruction 6055.1 and Headquarters, Department of the Army (HQDA) Letter 1-01-1 (2001), which identifies a list of chemicals of concern beyond traditional chemical warfare agents to include more common chemicals that could pose immediate or even delayed/long-term health impacts to deployed personnel[4]. Although there are U.S. tri-service (USACHPPM TG-230) standards for carbaryl and lindane in potable water, DoD forces currently lack the capability to detect these chemicals in field water supplies. The following proposal would achieve such results by combining SPME sampling with analysis using field-portable gas chromatography/mass spectrometry (GC-MS) analysis with the use of a low thermal mass (LTM), resistively heated column (RHC). By reducing the turn-around time for field water sample analysis, force health protection would be enhanced.

Research Goal

The goal of this research project is to develop a rapid sampling and analytical method for the detection of selected pesticides in environmental waters. The rapid identification and quantification of such chemical contaminants would be vital for the completion of an accurate environmental health assessment needed to determine the hazards associated with the chemicals of concern. Once a determination of the risk level is made, control measures can be developed and applied to reduce or eliminate the potential for adverse health effects. The principles and methods identified during this research can be readily applied to the identification of most pesticides in environmental waters. The timely and on-site information gained from the use of

such methods can reduce or eliminate the need to send samples to environmental labs outside military areas of operation. It could also be a feasible method of environmental sampling and analysis that can augment, or substitute, other methods used by public health agencies and environmental laboratories.

Research Question

Can field-portable gas chromatography using a low thermal mass resistively heated column, coupled to a mass spectrometer detector, rapidly and accurately detect and quantify varying concentrations of carbaryl and lindane in environmental waters using immersion solid phase microextraction (SPME) as an extraction method?

Specific Aims

The specific aims of this research were to: (1) Identify the SPME fiber that has the optimal characteristics required to extract each pesticide from water through direct immersion sampling, (2) Identify the optimal extraction parameters (extraction time and temperature) and matrix effects (ionic strength) for GC-MS analysis of laboratory grade pesticides (3) Determine the sensitivity of the fibers and method for a range of known concentrations of each pesticide spiked into samples using four different water sources that include: a control using ultra-pure deionized water produced from reverse osmosis filtration, a simulated post-production water that uses reverse osmosis filtered water with a chlorine residual, modeled ground water, and raw surface water, (4) Determine whether there are differences in the detection capabilities of lab grade GC-MS equipment and a field-portable GC-MS system with a low thermal mass resistively heated column while under field conditions.

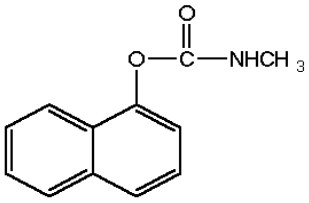
CHAPTER TWO: LITERATURE REVIEW

Carbaryl

Background

Table 2.1 shows carbaryl's chemical structure and physical properties. Carbaryl, commonly referred to as Sevin, is a carbamate class pesticide that is typically found as baits, dusts, granules, wettable powders and can also be used for aqueous dispersion[5]. Two forms of carbaryl (80% water dispersal powder (Sevin 80S) and 43.4% liquid) have been identified in the AFPMB Technical Guide (TG) 24, Contingency Pest Management Pocket Guide, as approved pesticides for use by DoD personnel inside and outside of the continental United States[1].

Table 2.1 - Carbaryl Structure and Properties

Carbaryl (CAS Number 63-25-2)	$C_{12}H_{11}NO_2$
Carbaryl (1-Naphthyl methylcarbamate (Trade Name: Sevin)	
Molecular Weight[6]	201
Water Solubility[6]	40
Vapor Pressure[7]	3.97×10^{-5} mm Hg at 25°C
Bioconcentration Factor[7]	9 - 34
Henry's Law Constant[7]	8.8×10^{-8} atm-cu m/mole
K_{ow}[6]	230
K_{oc}[7]	251

[6]Ney, Ronald E., Jr., Fate and Transport of Organic Chemicals in the Environment, A Practical Guide, 3rd Edition, Government Institutes, Inc., Rockville, MD, 1998

[7]Defense Occupational and Environmental Health Readiness System (DOEHRS)

Dietary Intake Values

Insufficient data is available to calculate the average daily intakes from water and air ingestion[7]. Food represents the major quantifiable source of carbaryl intake in the United States population. The average daily adult dietary intake for the years 1980-84 ranged from 0.12-0.032 μg/kg body weight/day. A single oral dose of 250 mg (about 2.8 mg/kg) has been shown to produce moderate illness in man; however, no subjective effects were noted when administered at doses of 0.13 mg/kg/day for six weeks[7]. The acceptable daily intake is 0.1 mg/kg/day with a maximum permissible intake of 6 mg/day[5].

Deployment Exposure Values and Toxicological Effects

USACHPPM TG-230 lists the established short-term ($\leq$ 5 days) and long-term ($\geq$ 2 weeks) military exposure guidelines (MEG) for water consumption during deployments. A range of MEGs is identified for likely consumption rates of 5.0 L/day and 15 L/day. Table 2.2 identifies the health effects associated with water-MEGs. For a consumption rate of 5.0 L/day, the short-term and long-term MEGs for carbaryl are identical at 1.4 mg/L. For a consumption rate of 15 L/day, the short-term and long-term MEGs are again identical at 0.5 mg/L[4].

Carbaryl is easily absorbed in humans if inhaled, ingested, or absorbed via dermal exposure, but has a low cumulative potential in tissue and blood. Exposure to concentrations that exceed the MEGs can potentially result in a wide range of symptoms and result in low to moderate mammalian toxicity. Since the inhibition of cholinesterase is the principle mechanism of action, the clinical symptoms include: increased bronchial secretion, excessive sweating, salivation, pinpoint pupils, bronchoconstriction, abdominal cramps (vomiting and diarrhea), bradycardia, fasciculation of fine muscles, tachycardia, headache, dizziness, anxiety, mental confusion, convulsions, coma, and depression of the respiratory center. Carbaryl is eliminated from the

body as a result of hydrolysis, which results in the production of 1-naphthol (the principle metabolite in humans), carbon dioxide, and methylamine[7].

Table 2.2 - USACHPPM TG-230 Definitions of Health Effects Associated with Water-MEGs

Exposure Duration		Health Effect	Health Effects and Performance Degradation*
Short-Term	5 days 5 or 15 L/day	Minimal To Non-significant	The drinking water concentration for a continuous daily consumption of either 5 L/day or 15 L/day for up to 5 days that should not impair performance and is considered protective against significant non-cancer effects. Increasing concentrations and/or duration could result in performance degradation, need for medical intervention, or increase the potential for delayed/permanent disease (i.e., kidney disease or cancer).
Short-Term	14 days 5 or 15 L/day	Minimal To Non-significant	The drinking water concentration for a continuous daily consumption of either 5 L/day or 15 L/day for up to 14 days that should not impair performance and is considered protective against significant non-cancer effects. Increasing concentrations and/or duration could result in performance degradation, need for medical intervention, or increase the potential for delayed/permanent disease (i.e., kidney disease or cancer).
Long-Term	1 year 5 or 15 L/day	Non-significant To None	The drinking water concentration for a continuous daily consumption of either 5 L/day or 15 L/day for up to 1 year that should not impair performance and is considered protective against health effects including chronic disease and increased risk to cancer (i.e., cancer risk greater than 1×10^{-4}). Increasing concentrations and/or duration could increase the potential for delayed/permanent disease (i.e., kidney disease or cancer).

* Sensitive individuals may be predisposed to toxic effects and, therefore, maybe more susceptible
[4]USACHPPM Technical Guide 230, Chemical Exposure Guidelines for Deployed Military Personnel, January 2002

Environmental Fate and Transport

Carbaryl's production and use as a pesticide is expected to result in its direct release to the environment. If released into air, carbaryl's vapor pressure indicates it will exist in both the vapor and particulate phases in the ambient atmosphere. Carbaryl's vapor-phase will be degraded by reactions with photo-chemically produced hydroxyl radicals with a half-life estimated at fifteen hours. The particulate phase is likely to be removed from the atmosphere by wet and dry deposition. Photolysis half-lives have been calculated at a range of 52-264 hours for a summer day at latitude 40 degrees north[7].

If released to soil, carbaryl is expected to have moderate mobility based on its K_{oc} value. Carbaryl is expected to slowly photolyze on surface soil at a rate dependent on the water content. Volatilization from moist soil surfaces is not expected to be an important fate process based on the estimated Henry's Law constant. Carbaryl is not expected to volatilize from dry soil surfaces based on its vapor pressure[7].

If released into water, carbaryl is not expected to adsorb to sediment and suspended solids in water based on its K_{oc}. Volatilization from water surfaces is not expected to be an important fate process based upon its Henry's Law constant. At 20°C, hydrolysis half-lives in water are 10.5 days, 1.8 days, 2.5 hours, and 15 minutes at pH values of 7, 8, 9, and 10 respectively. Bioconcentration factor (BCF) values range from 9-34, which suggests bioconcentration in aquatic organisms is low[7]. Carbaryl's water solubility also indicates that leaching into soil and/or runoff could occur, but is not likely. The K_{ow} value indicates that accumulation should not occur[6].

Environmental Water Concentrations

In surface water sources, carbaryl has been detected at a concentration of 3.0 μg/L in stream water adjacent to a land spraying area in Canada five days following an application at a rate of 280 g/ha. In a one-year study from 1993-94, a total of twenty-five water samples were taken at the mouths of two tributary streams of the South Platte River in Colorado and studied for pesticide concentrations. The tributary that originated from an agricultural region contained carbaryl ranging from <0.046-1.5 μg/L while the tributary that originated from an urban setting contained carbaryl ranging from 0.15-2.5 μg/L. Carbaryl detection from the agricultural tributary occurred during seasonal spraying periods of bean crops while consistent detection

occurred from the urban tributary as a result of repeated applications for residential and commercial insect control[7].

In groundwater, carbaryl has not been detected in farm wells (detection limit of 1.0 μg/L) where the land was treated with the pesticide. However, it has been detected (concentration not specified) in groundwater samples in Solano and Ventura counties of California at a concentration ranging from 10-55 μg/L. Carbaryl has also been detected in coastal fog and air samples collected from locations along the Pacific coast near Monterey, CA and found at concentrations ranging from 0.069-4.0 μg/L. Carbaryl has been detected, but not quantified, in drinking water[7].

Regulations and Standards

Carbaryl is designated as a hazardous substance under section 311(b)(2)(A) of the Federal Water Pollution Control Act and further regulated by the Clean Water Act Amendments of 1977 and 1978. These regulations apply to discharges of the pesticide as well as any solutions and mixtures containing carbaryl. According to 40 CFR 261.33, when carbaryl becomes a waste, it must be managed according to federal and/or state hazardous waste regulations. The Occupational Safety and Health Administration's (OSHA) permissible exposure limit (PEL) for an eight-hour time weighted average (TWA) exposure, the National Institute of Occupation Safety and Health's (NIOSH) recommended exposure limit (REL) for a ten-hour TWA exposure, and the American Conference of Governmental Industrial Hygienist (ACGIH) threshold limit value (TLV) for an eight-hour TWA exposure are all 5.0 mg/m^3. The immediately dangerous to life or health (IDLH) value has been set at 100 mg/m^3 [7].

Standard Analytical Methods

Typical analysis for carbaryl in the environment has followed EPA Method 8270B-W (GC-MS with capillary columns) and EPA Methods 531 and 8318-W (High Performance Liquid Chromatography (HPLC) with post column derivatization). EPA Method 8270B-W is used to analyze semivolatile organic compounds in various types of extracts including groundwater. For liquid samples, a 1.0 L sample is required and adjusted to pH < 2 with sulfuric acid and 1.0 mL of a surrogate standard mixture is added to it to assist in determining the quantity of analyte lost during the extraction process. The sample is extracted with methylene chloride in a continuous extractor for 18-24 hours. The aqueous phase is adjusted to a pH > 11 with sodium hydroxide and the sample is re-extracted with methylene chloride for another 18-24 hours. The extract is dried by passing it through a column of anhydrous sodium sulfate and then concentrating it to 1.0 mL. The concentrated extracts are then analyzed by capillary GC-MS[5,7].

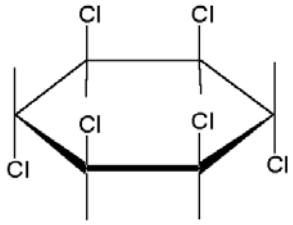
EPA Methods 531 and 8318-W are methods used to determine the presence of various carbamate-class pesticides in finished drinking water and groundwater. In both methods, samples are adjusted to pH 3.0 ± 0.2 with 2.5 M monochloroacetic acid buffer and an internal standard is added. A 5.0 mL aliquot is filtered using a syringe and 400 mL of the sample is analyzed using HPLC with gradient elution. After elution from the HPLC column, the analytes are hydrolyzed with 0.05 N sodium hydroxide at 95ºC in a post column reactor. The methyl amine formed during hydrolysis is reacted with o-phthalaldehyde and 2-mercaptoethanol to form a highly fluorescent derivative, which is detected with a fluorescence detector[5,7].

Lindane

Background

Table 2.3 lists the chemical structure and physical properties of lindane, or gamma-benzenehexachloride (γ-BHC). Lindane is an organochlorine class pesticide that has been heavily regulated in the United States in the past two decades due to its ability to persist in the environment. Lindane is defined as not less than ninety-nine percent of the pure gamma isomer of hexachlorocyclohexane (HCH) and is also known as gamma-benzene hexachloride (gamma-BHC) through common use. Gamma-BHC is actually a complex mixture of six related isomers[7]. No form of lindane is approved for DoD use[1].

Table 2.3 - Lindane Structure and Properties

Lindane (CAS Number 58-89-9)	$C_6H_6Cl_6$
Lindane [Gamma isomer, benzene hexachloride (BHC)]	
Molecular Weight[6]	290.8
Water Solubility[6]	0.15
Vapor Pressure[7]	5.57×10^{-5} mm Hg at 25°C
Bioconcentration Factor[7]	5.5 – 2100
Henry's Law Constant[7]	3.5×10^{-6} atm-cu m/mole
Log K_{ow}[7]	3.72
K_{oc}[6]	911

[6]Ney, Ronald E., Jr., Fate and Transport of Organic Chemicals in the Environment, A Practical Guide, 3rd Edition, Government Institutes, Inc., Rockville, MD, 1998

[7]Defense Occupational and Environmental Health Readiness System (DOEHRS)

Dietary Intake Values

Ingestion of contaminated food or water would be the most likely route of exposure for lindane, but exposure by inhalation or absorption through the skin may also occur[8]. The major potential dietary sources of lindane include milk, eggs, dairy products, and seafood. The average dietary intake of lindane and its isomers by the U.S. population has not been quantified, but is estimated to be only at trace quantities and is undergoing a significant, steady decline[9].

Deployment Exposure Values and Toxicological Effects

USACHPPM TG-230 lists MEGs for lindane. For a consumption rate of 5.0 L/day, the short-term and long-term MEGs are identical at 0.6 mg/L. For a consumption rate of 15 L/day, the short-term and long-term MEGs are identical at 0.2 mg/L. Exposure to concentrations that exceed these MEGs can potentially result in a wide range of symptoms that include: irritability, restlessness, insomnia, anxiety, dizziness, malaise, headache, nausea, fever, cyanosis, vomiting, and loss of muscle coordination. Higher exposures can result in muscle spasms, seizures, and convulsions. Increased susceptibility to nervous system changes may occur at concentrations between 0.6 and 3.5 mg/L[7]. Signs of poisoning begin to develop at 3.5 mg/L[4]. The lethal dose is approximately 125 mg/kg. The ACGIH lists lindane as a class A3 carcinogen (confirmed animal carcinogen with unknown relevance to humans)[7].

Environmental Fate and Transport

Lindane typically enters the environment as a result of runoff from agricultural land, from use in the forestry industry, and from home and garden applications. When lindane enters the atmosphere, rain-out and dry deposition are likely to be important fate processes with an overall estimated atmospheric retention time of 17 weeks. Photo-chemically produced hydroxyl radicals react with and degrade vapor-phase lindane. Lindane adsorbed to sediments may be released

back into the atmosphere in gas bubbles from the methanogenesis and denitrification processes of bacteria. Photodegradation is not expected to be a significant removal process for atmospheric lindane. Based on its vapor pressure, lindane is expected to exist almost completely in the vapor phase in the ambient air[7].

When lindane is released to water, it is likely to dissolve and remain in the water column of surface waters despite its low water solubility[7]. It is also expected to adsorb to sediments and is not expected to volatilize significantly. If released to acidic or neutral waters, it is not expected to hydrolyze significantly. However, in basic water (pH > 9), significant hydrolysis may occur[8]. The importance of photolysis as a fate for lindane in water is unclear, but half-life estimates have been reported in the range of 169 hours at pH 9.3 to 1540 hours at pH 7.8. Hydrolysis may be another fate for lindane in water. Half-lives as a result of hydrolysis in surface waters have been estimated in a range from 13.8-240 days and 5.9-240 days in ground water[7].

Transport to sediment should be slow and result predominantly from diffusion rather than settling. However, when released to soil, lindane will most likely volatilize and/or slowly leach to groundwater[8]. Lindane may slowly biodegrade in aerobic media and will rapidly degrade under anaerobic conditions. Half-lives have been estimated in the range of 31-413 days under aerobic conditions and 5.9-30 days under anaerobic conditions[7].

Regulations and Standards

The Environmental Protection Agency (EPA) regulates lindane and its isomers under the Clean Water Act (CWA), the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the Safe Drinking Water Act (SDWA), and the Resource Conservation and Recovery Act (RCRA). Lindane is registered under FIFRA for use as a seed and seedling treatment. Proposed and

established effluent guidelines under the CWA and CERCLA control the release of lindane from several point sources. A maximum contaminant level (MCL) and a maximum contaminant level goal (MCLG) of 0.2 µg/L have been proposed for lindane in drinking water under the SDWA. The Food and Drug Administration (FDA) regulates the presence of lindane in drinking water and drugs and prohibits its residues in animal feed. The ACGIH recommends a TLV of 0.5 mg/ m^3 with a skin notation indicated. NIOSH has established a REL of 0.5 mg/ m^3as a 10-hour TWA with a skin notation. OSHA adopted a PEL of 0.5 mg/ m^3as an 8-hour TWA[9].

Standard Analytical Methods

EPA methods 508, 525, 608, and 8081 are typically used for the analysis of lindane in the environment. EPA Method 508 requires a sample of approximately 1.0 L that is solvent extracted with methylene chloride by shaking it in a separatory funnel, or through mechanical tumbling in a bottle. The methylene chloride extract is isolated, dried, and concentrated to a volume of 5.0 mL after solvent substitution with methyl tert-butyl ether (MTBE). EPA Method 525 uses liquid-solid extraction (LSE) to extract organic compound analytes from water samples. Extraction occurs by passing one liter of sample water through a cartridge that contains one gram of a solid inorganic matrix coated with a chemically bonded Cl_8 organic phase. Organic compounds are eluted from the LSE cartridge with a small quantity of methylene chloride and concentrated further by evaporation of the solvent. EPA Method 608 requires a sample of one liter that is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a final volume of 10 mL or less. EPA Method 8081 requires a sample of either 1.0 L for liquids or 2.0 to 30 g for solids. Liquid samples are extracted at neutral pH with methylene chloride using either a separatory funnel, or a continuous liquid-liquid extractor. Solid samples are extracted with

hexane-acetone (1:1), or methylene chloride-acetone (1:1), using soxhlet extraction, automated soxhlet extraction, or ultrasonic extraction. A variety of cleanup steps may be required for the extract depending on the nature of the co-extracted matrix interferences and/or the target analytes. Separation and measurement of analytes in prepared extracts for each method are accomplished by injecting a 1.0 to 4.0 μL aliquot into a gas chromatography (GC) system with either an electron capture detector (ECD), or a mass selective detector (MSD)[8].

Traditional Extraction Methods

Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are two traditional methods used for the extraction and separation of pesticides from environmental waters. These methods are suitable for use with aqueous matricies and provide the capability for detecting pesticides at low concentrations. However, they tend to be complex, tedious, and do not permit rapid analysis in a field setting. The EPA sample preparation methods for both carbaryl and lindane require large quantities of hazardous solvents, which can produce significant background interferences in spectra analysis. Pre-concentration of the extract prior to analysis is also required, which greatly increases the total time of analysis[10].

LLE is a separation process that uses two immiscible phases to separate a solute and drive it from one phase into the other. Separation is accomplished by the addition of a second solvent to a liquid sample that has a greater affinity to bind with the target analyte. The two solvents flow in opposite directions. The lighter solvent flows upward while the heavier flows downward. The substance to be separated is in contact with both solvents and is dissolved in each stream according to a ratio determined by the distribution coefficient. As the immiscible solvents

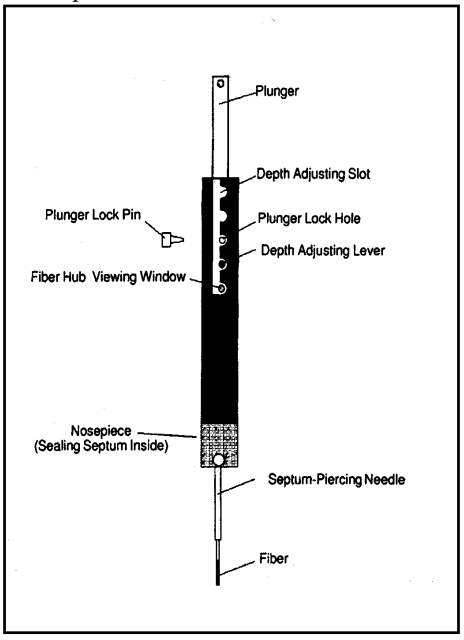
separate, the target analyte moves towards and dissolves into the solvent for which it has a greater binding affinity, causing separation and concentration into the one solvent[11].

SPE is a procedure for isolating target organic analytes from aqueous samples using solid-phase extraction media. Sample preparation procedures vary by analyte group. Extraction of some groups requires that the pH of the sample be adjusted to a specified value prior to extraction. Following any necessary pH adjustment, a measured volume of sample is extracted by passing it through the solid-phase extraction disks or cartridges, which are held in an extraction device designed for vacuum filtration of the sample. Target analytes are eluted from the solid-phase media using an appropriate solvent, which is collected in a receiving container. The resulting solvent extract is dried using sodium sulfate and concentrated as needed into a solvent-compatible extract for measurement of the target analyte[12].

Solid Phase Microextraction

Solid Phase Microextraction (SPME) is a solvent-free technique that can be used for the extraction of pesticides from environmental waters. It integrates extraction from the liquid matrix, concentration, and sample preparation into a single step. Figure 2.1 shows a field portable SPME device and its two major components: the syringe assembly and fiber assembly. The syringe acts as a holder for the fiber assembly, which is comprised of a needle that protects a small-diameter fused-silica fiber that is coated with a liquid polymeric stationary phase. SPME

Figure 2.1 - SPME Portable Field Sampler with fiber[13].



fibers possess either absorptive or adsorptive characteristics. Absorption is a non-competitive process that does not result in the complete extraction of an analyte from a sample matrix unless the concentration of the analyte is extremely low and has a very high affinity for binding to a specific fiber. Absorption results in the analyte partitioning into the SPME fiber's liquid coating. Adsorption is a competitive process where analytes compete for pore binding sites on the surface of the SPME fiber. The size of the pore space enhances the sensitivity for some analytes based on their molecular size. Adsorptive fibers use a mixed phase system containing a solid polymer particle that is blended into the liquid phase[14].

By understanding the chemical characteristics of a specific analyte, and knowing the binding properties of different SPME fibers, it can be determined which fiber is most appropriate for the extraction of pesticides from a sample matrix. SPME fibers are specifically suited to certain chemicals based on the compound's polarity (polar, semi-polar, and non-polar) and are manufactured with either singe liquid-phase coating, or with mixed-phase coatings that blend solid polymer particles into the liquid-phase. The polydimethysiloxane (PDMS) and polyacrylate (PA) fibers have single-phase coatings and the carbowax/carboxen fibers have mixed phase coatings. PDMS fibers are versatile single-phase fibers that vary in liquid-phase thickness (7, 30, and 100 μm) and have the ability to extract a wide range of analyte concentrations in a sample matrix. PDMS fibers have many advantages that include having very robust liquid coatings that are able to withstand injector temperatures up to 300°C and are good for the extraction of non-polar analytes since it is composed of a non-polar phase. A PDMS fiber would be an appropriate choice for the extraction of lindane, which is non-polar. However, it can also be applied successfully to more polar compounds, particularly after optimizing the extraction conditions from the sample matrix. PA fibers (85 μm phase thickness) are useful for

the extraction of more polar compounds, which potentially makes it better suited for extraction of more polar organophosphate pesticides, or even a weakly polar pesticide like carbaryl. It is a low-density solid polymer at room temperature that allows analytes to diffuse into the fiber coating, but with a lower diffusion coefficient compared to the PDMS. The 65 µm PDMS/divinylbenze (DVB) fiber, the 70 µm CW/DVB fiber, and the 85 µm Carboxen/PDMS fiber are mixed-phase fibers that are usually more suitable for volatile compounds and have complimentary properties compared to the other two single-phase fiber types. The mixed phase fibers are known to have less adsorption discrimination as a function of an analyte's molecular weight[15].

During extraction of an analyte from a liquid sample, the coated fiber is either directly immersed into the aqueous phase of the sample, or held in the headspace (HS) above the sample. These techniques allow analytes to partition to the fibers in accordance with their affinity toward the fiber's coating. HS sampling involves the partitioning of the analyte between the aqueous and gaseous phases and is especially suitable for analytes that are of a volatile, or semi-volatile, nature because many of these compounds can easily diffuse into the sample headspace from the liquid matrix. However, due to the physical characteristics (high molecular weight, low volatility) of carbaryl and lindane, immersion sampling is the better option for this study[10,16].

Low Thermal Mass (LTM) Resistively Heated Column (RHC)

One critical outcome of STO IV.ME.1999.01 was to develop equipment and methods that could achieve a total analysis time of no more than 30 minutes. Long extraction periods may be required for trace levels of pesticides in liquid samples; therefore, shortening the GC analytical time can reduce the total time of analysis. Temperature programming speed is limited by the rate

at which a column can be heated. The time to complete GC-MS analysis can be shortened with the use of a LTM resistively heated capillary column that allows for rapid temperature ramping (up to 20ºC per second). A conventional GC oven ramps slowly (about 2ºC per second), which results in a longer total time of analysis. The LTM column is well suited for this task since it heats very rapidly with the assistance of a heating wire element that is coiled in with a standard GC capillary column and encased in a foil casing. However, separation efficiency and peak resolution may decrease with a LTM/RHC as a result of the increased temperature ramping rates. Cycle times (total time for a run to include cooling prior to a subsequent run) also depend on how quickly the column can cool. The LTM design combines a temperature-sensing element with the GC column along with a small fan that is mounted in direct proximity to the encased column. This configuration allows the column module to cool very quickly and provide fast analytical cycle times. Overall, the chromatography obtained using LTM assemblies has been studied by a number of investigators and has been shown to provide temperature programming performance equivalent to that obtained with standard laboratory ovens[17].

CHAPTER THREE: METHODS

Materials

Stock Solutions

Laboratory grade pesticides that are at least 97% pure were obtained from Sigma-Aldrich (St. Louis, MO) and used throughout this study. Stock solutions were prepared and stored in class A rated Kimax® brand volumetric flasks (10, 100, 250, 500, and 1000 mL) with pennyhead stoppers (Fisher Scientific, Suwanee, GA). A Sartorius BP 61S digital analytical balance with monolithic weighing cell and glass draft shields (Sartorius Group, Goettingen, Germany) was used to weigh all pesticides in solid form. Pesticides were diluted with 99% pure methanol (Sigma-Aldrich, St. Louis, MO). Ultra-pure water produced with a Millipore® MilliQ deionized reverse osmosis water purification system (Milford, MA), installed at the Armed Forces Radiobiology Research Institute (AFRRI), was used to further dilute pesticides. Magnetic stirring bars (Sigma-Aldrich, St. Louis, MO) were used to mix solutions on a magnetic stir plate with heater (IKA Works, Inc., Wilmington, NC).

SPME Fiber Selection/Optimization

All SPME fibers and holders were purchased from Supelco (Bellefonte, PA). Fifteen and twenty milliliter clear glass vials with open screw top closures fitted with polytetrafluoroethane (PTFE)-lined silicone speta (Supelco, Bellefonte, PA) were used for manual extractions. Twenty mL clear glass vials with crimp-on AlumiTin caps fitted with a penetrable Teflon/Blue silicone septa (MicroLiter Analytical Supplies, Inc., Suwanee, GA) were used when using the auto-sampler. Magnetic stir bars were used in all vials for continuous stirring during equilibration and extraction. A Nalgene polypropylene volumetric transfer pipet, with 10 mL chemical resistant glass pipets (Fisher Scientific, Suwanee, GA), was used to transfer stock solutions to vials.

Optimization of Extraction Parameters and Matrix Effects

The materials used to prepare samples for this phase were identical to those used for SPME Fiber Selection/Optimization. Sodium chloride that is at least 99% pure (Fisher Scientific, Suwanee, GA) was used to adjust the ionic strength of solutions.

Water Source Comparison – Fiber and Method Sensitivity

The materials used to prepare samples for this phase were identical to those used in the first two phases. Water from various sources was collected, transported, and stored in Qorpak 3.8L amber safety-coated wide-mouth glass bottles (Fisher Scientific, Suwanee, GA). Ultra-pure water produced with a Millipore® MilliQ deionized reverse osmosis water purification system (Milford, MA, USA) installed at the Armed Forces Radiobiology Research Institute (AFRRI) was used as a control and for the simulated post-production water that was spiked with granular (66.8% available chlorine) calcium hypochlorite (Fisher Scientific, Suwanee, GA). Raw surface water was obtained from an area pond on the grounds of the National Naval Medical Center, Bethesda, MD. Modeled ground water was prepared by dissolving sodium chloride (Fisher Scientific, Suwanee, GA) and anhydrous sodium sulfate (Sigma-Aldrich, St. Louis, MO) in ultra-pure water.

Gas Chromatography/Mass Spectrometry Methods

Analysis to select an optimal fiber, optimize the extraction method, and determine the sensitivity of these parameters was initially performed on a laboratory-grade Agilent 6890 gas chromatograph with a 5973 quadrapole mass selective detector (Agilent Technologies, Wilmington, DE,). The GC was fitted with a DB-1, 30 m × 0.25 mm ID × 1 μm phase column (J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas and held constant at a

rate of 1.9 cm/sec. Desorption of the SPME fiber samples was accomplished in the splitless injection mode for 1.0 min, followed by a 55 mL/min injector purge. A 0.75 mm deactivated glass injection port liner (Supelco, Bellafonte, PA, USA) was used to rapidly transfer desorbed analytes onto the front of the column. The oven was initially programmed to hold at 40°C for one minute, ramped up at 20°C per minute to 300°C, and held for 5.0 minutes for a total run time of 19 minutes. Fibers were extended into the injection port for 10 minutes to allow complete desorption of analytes from the fiber coating. The injector temperature was set at 260°C and the mass spectrometer transfer line was kept at 280°C. Electron impact ionization (70 eV) was used and mass spectra were collected over the range of 10-250 *m/z* (mass-to-charge ratio). Sample retention characteristics and mass spectra were stored using the Agilent Chemstation software package.

Automated sampling was carried out with a CTC CombiPAL LEAP GC sampler (LEAP Technologies, Inc., Carrboro, NC). After sample preparation, vials were placed on the CombiPAL sample rack. Each sample was allowed to equilibrate for ten minutes by being individually introduced to the instrument's sample heater/magnetic stir box for heating and stirring. Following the equilibration period, SPME fibers were automatically introduced to the sample's aqueous matrix for analyte extraction. SPME fibers were automatically introduced into the Agilent 6890's injection port for desorption and analysis following similar guidelines previously stated. Individual methods and runs were introduced to the 6890 for GC-MS analysis through the CombiPAL software.

A field-portable Viking Spectra Trak 573 GC system (Bruker Daltonics, Billerica, MA), matched to a 5973 MS detector, was employed for the field-testing component. The MS section of this instrument is based on the Hewlett Packard 5973 ion source and monolithic quadrupole

mass filter. The instrument was equipped with a deactivated injection port liner (0.75 mm I.D., Supelco, Bellefonte, PA) designed for thermal desorption of analytes from a SPME fiber and a 30 m × 0.25 mm × 0.25 μm DB-5 low thermal mass (LTM) resistively heated column (RHC) from RVM Scientific (Santa Barbara, CA). High purity helium was used as the carrier gas with a column head pressure of 17 pounds-per-square-inch (psi) and an initial linear velocity of 47 cm/s. The oven was initially programmed to hold at 40°C for five seconds and then ramp at 150°C per minute to 300°C and hold for 271 seconds for a total run time of six minutes. The injector temperature was maintained at 260°C throughout and the mass spectrometer transfer line was kept at 260°C. Electron impact ionization (70 eV) was used and mass spectra were collected over the range of 10-250 *m/z* (mass-to-charge ratio). Sample retention characteristics and mass spectra were stored using the Agilent Chemstation software package.

SPME Sampling

In order to determine whether immersion SPME sampling coupled to GC-MS analysis with a LTM/RHC can rapidly detect trace quantities of pesticides in environmental water samples, methods for the detection of carbaryl and lindane were developed and optimized. Each method provided qualitative and quantitative identification and measurement of target compounds.

Stock Solutions

For carbaryl, 1.0 L stock solutions at a concentration of 100 mg/L (1.0 mg/L = 1.0 ppm) were prepared for SPME fiber selection/optimization and for optimization of extraction parameters and matrix effects. For lindane, a stock solution of 100 mg/L was used for SPME selection/optimization and a 15 mg/L solution was used for the optimization of extraction parameters. Two hundred fifty (250) mL stock solutions were prepared for water source

comparison at concentrations of 50 mg/L and 100 mg/L. Separate stock solutions were prepared for each pesticide. The pesticides were purchased in a solid form and were dissolved in methanol, which improved their solubility in water and uniform dispersion in solution. Methanol accounted for one percent of the total solution volume. For 1.0 L stock solutions, at a concentration of 100 mg/L, 0.1 g of pesticide was added to a 10 mL volumetric flask and filled to a total volume of 10 mL with methanol. To completely dissolve the pesticide, a magnetic stir bar was added to the flask and stirred on a magnetic stir plate at 1200 rpm for approximately 2.0 – 3.0 minutes. This solution was added to a 1.0 L volumetric flask and filled with ultra-pure water to a total volume of 1.0 L. A magnetic stir bar was added to the solution and stirred on a magnetic stir plate at 200 rpm for one hour prior to use. For 250 mL stock solutions at concentrations of 50 mg/L and 100 mg/L, 0.0125 g and 0.025 g of pesticide were added to 2.5 mL of methanol respectively in 10 mL volumetric flasks and stirred until completely dissolved in a similar fashion to other stock solutions. These solutions were added to 250 mL volumetric flasks and filled to a total volume of 250 mL with ultra-pure water. Magnetic stir bars were added and solutions were stirred for one hour at 200 rpm prior to use. All stock solutions were stirred while at room temperature. Following daily use, all solutions were stored at 4°C ($\pm$ 2°C) for no more than 15 days and stirred for one hour prior to use each day.

SPME Fiber Conditioning and Carryover

SPME fibers required conditioning prior to first use in accordance with manufacturer recommendations. Conditioning was accomplished by extending a fiber into the injection port of the gas chromatograph (GC) for a set time (0.5 - 4.0 hours) and at a set temperature (250°-300°C). Blank runs on each fiber were performed each day prior to the first set of runs to ensure contaminants from the ambient environment that may have partitioned onto the fiber were

removed. Fibers were checked after 3-5 sets of subsequent runs to determine whether residual analyte was being totally desorbed from the fiber, or whether it was being carried over to subsequent runs. A blank run was used to determine the presence of carryover. If pesticide analyte was detected, the amount present was considered to be the amount of carry over. If this occurred, all remaining analyte were thermally desorbed from the fiber prior to performing additional runs. This was accomplished with the use of a SPME conditioning device, or by allowing a fiber to remain extended in the GC injection port for a sufficient length of time until no carry over was observed[15].

SPME Fiber Selection/Optimization

Conditions

Prior to the development and optimization of methods for the extraction of carbaryl and lindane from environmental waters, an optimal SPME fiber was selected that was best suited for the extraction of each compound. Fiber selection was accomplished using the Agilent 6890 GC, coupled to the CTC CombiPal auto-sampler, which used a 23-gauge SPME fiber holder. Seven commercially available fibers that were compatible with this system were evaluated and included the 7, 30, and 100 μm PDMS fibers, the 85 μm PA fiber, the 65 μm PDMS-DVB fiber, the 70 μm CW/DVB fiber, and the 85 μm Carboxen/PDMS fiber (Supelco, Bellafonte, PA). Each pesticide was examined independently. Triplicate samples were completed for each fiber to determine reproducibility and which fiber allowed the greatest amount of analyte to partition into the fiber coating and subsequently be desorbed into the analytical instrument.

Methods

Twenty (20) mL vials were filled with 18 mL of stock solution, which is the auto-sampler manufacturer's recommended volume for immersion sampling. All vials for an entire set of runs were filled at the same time and remained sealed at room temperature prior to equilibration and extraction. Each sample was allowed to equilibrate for ten minutes prior to extraction. During this period, samples were brought to 50°C in the auto-sampler's built in hot-block and agitator (LEAP Technologies Inc., Carrboro, NC), while being stirred at a rate of 750 rpm (software upper limit for CTC CombiPal). Fifty (50) degrees celsius and 750 rpm were maintained for the 30-minute extraction period that immediately followed equilibration. Immediately prior to extraction, a vial septum was pierced with the SPME fiber assembly. To avoid a wicking effect that could result in carry-over, the end of the needle assembly was not immersed in the liquid matrix, but held in the headspace approximately 2-3 mm above the liquid. The fiber was then extended out of the assembly sheath 30 mm (software maximum limit) into the liquid matrix. At the end of the extraction period, the SPME fiber was retracted into its protective sheath, removed from the vial, and immediately introduced into the heated GC injection port. GC-MS analysis commenced once the fiber was lowered into the injection port's midrange region (approximately 54 mm).

Statistical Analysis

The analyte peak areas for the three samples in each set were compared for reproducibility. This was accomplished by determining the relative standard deviation (RSD) for each set. RSDs were established by dividing the sample standard deviation by the sample mean and multiplying by 100 to obtain a percent of variation among individual samples in each data set[19]. The RSD described the variability of each individual sample's peak area in relation to the mean peak area

of that set. The variation in peak areas among fibers and within fibers was examined to determine whether the associated values were attained due to the dynamics of the fiber, or whether they occurred as a result of error. An initial screening suited for this purpose was accomplished using univariate analysis of variance (ANOVA). ANOVA helped to determine whether there were similarities or differences between the fibers. If the overall F-statistic from ANOVA was determined to be statistically significant, then it was concluded that the mean peak areas obtained from each different fibers were statistically different. It could then be concluded that the differences were likely to be a result of the dynamics of the fibers, rather than random error. The residuals from these ANOVA models were examined for normality, which is an assumption of ANOVA[19]. By plotting the residuals as a histogram with a normal curve overlaid, it could be determined whether the data appeared to be normally distributed. To confirm a normal distribution, the standardized residuals were graphed to determine whether the data lay between ± 2 standard deviations. If the data was highly skewed to one side of the mean, or many data points laid outside of ± 2 standard deviations, then it could have been log-transformed to try to normalize the data for further comparisons. The sets of values under comparison could then be examined to determine which specific fibers were similar or different. Tukey's *Post Hoc* analysis was used for this purpose, which produced pair-wise comparison values of each fiber. If ANOVA determined that the fibers were truly different, and Tukey's *Post Hoc* analysis showed specifically which fibers differed, then the fiber that produced the highest mean peak area was chosen as the optimal fiber for the pesticide under investigation.

Optimization of Extraction Parameters and Matrix Effects

Conditions

SPME is a method dependent on the equilibrium process involving the partitioning of an analyte from a liquid matrix into the stationary phase of the SPME fiber. The amount of analyte extracted depends on the mass transfer of the analyte through the aqueous phase, which is affected by stirring, extraction time, matrix temperature, and the solution's ionic strength.

For immersion sampling, the appropriate stir rate for sample preparation and extraction should be just below the point where a vortex is formed in the liquid sample. If headspace sampling were required, the rate of stirring would be a more important factor for driving the analyte into the sample headspace[15]. However, since this study was focused on immersion sampling, and the maximum stir rate of the CTC CombiPal autosampler did not produce a vortex in either the 15 mL or 20 mL vials, the rate of stirring was not optimized. All samples were stirred at the auto-sampler software's maximum limit throughout the study. The final phase of the study involved manual extractions under simulated field-conditions and an identical stir rate was used using a field-portable hot plate with magnetic stirrer.

The time the fiber was exposed, and the temperature of the aqueous matrix, were important parameters that needed to be optimized to achieve the greatest peak area for each analyte during analysis. Obtaining optimal extraction parameters was especially important to ensure effective extractions from environmental waters in which pesticides are present at extremely low concentrations[19]. A range of each of these parameters was examined above and below the initial points chosen to determine the optimal conditions. The initial settings chosen were based on estimates of the optimal points identified from previous research found in peer-reviewed journals[20,21,22,23,24,25,26].

The amount of analyte extracted by a fiber could be increased if the solubility of the analyte in water was decreased. Improving extraction efficiency could be achieved by altering the ionic strength of solutions with the addition of salt, or by adjusting the pH of the water. Based on similar research found in peer-review journals, altering the pH of the liquid matrix had little or no effect on partitioning analyte into a fiber's coating[27,28]. Therefore, pH was not optimized during this study. The alteration of a solution's ionic strength, a procedure referred to as salting, was accomplished with the addition of salts like sodium chloride (NaCl) or sodium sulfate (Na_2SO_4). Salting could increase or decrease the amount of analyte extracted, depending on the compound and salt concentrations. Substantial increases in analyte extraction have been shown to occur at salt concentrations above 1% saturated weight-per-volume (w/v), which could lead to about an order of magnitude increase in sensitivity at the 30% saturated level. Saturation with salt can be used to lower the detection limits of an analyte in a sample matrix and help to normalize random salt concentration in natural matrices[15]. Ultimately, this contributes to enhanced partitioning onto the SPME fiber, which may be necessary to extract and detect trace amounts of polar and non-polar pesticides in aqueous matrices[16].

Methods

Extraction Temperature. Extraction temperature was optimized first since it played the most important role in the extraction process for controlling the diffusion rate of analytes into a fiber's coating[16]. All samples were prepared in a similar manner as previous phases of this study. The effect of temperature in the extraction yield was evaluated by varying the sample matrix temperature between 30ºC and 100ºC in increments of 10 degrees. The range of temperatures to be investigated represented the allowable range of the CTC CombiPal auto-sampler software package. All samples were allowed to equilibrate for 10 minutes while being

stirred at 750 rpm at the temperature under investigation. Identical conditions were maintained during the 30 minute extraction period that immediately follows the equilibration period. GC-MS analysis commenced immediately following the extraction period.

Extraction Time. Since pesticides are often found in trace quantities in environmental samples, the extraction time is an important parameter to optimize to allow sufficient time for analytes to partition into a fiber's coating for analysis. For analytes with high molecular masses, such as the pesticides used in this study, the time for a fiber to reach equilibrium may vary greatly. If this method were to be used as a rapid field screening method, 30 minutes or more would likely be too lengthy of an extraction time. Therefore, the time selected to perform the sample analysis may not be the time necessary for the fiber to reach equilibrium. Choosing an extraction time that is less than the equilibrium time may affect the sensitivity and precision of the method. However, even if fiber equilibrium is not reached, it is not likely that the overall sensitivity of the method will be significantly affected for the compounds under investigation[13,16]. Since the initial extraction time chosen was 30 minutes, the range that was evaluated was 1, 5, 15, 30, 45, 60, 90, 100 (maximum for CombiPal software) minutes. All samples were prepared and handled in a similar manner as previous work in this study; however, the optimal temperature determined from the previous step was used for the remainder of the study.

Ionic Strength (Salting). The addition of salt to adjust the ionic strength of the solution was accomplished by the addition of NaCl. At 20°C, the saturation level of NaCl in water is 360 g/L[29]. Ionic strengths of 0, 10, 20, 30, 40, and 100 percent saturation were evaluated. One hundred (100) mL stock solutions were prepared for each pesticide. 0% saturation was used as the control for comparison purposes. 3.6 g, 7.2 g, 10.8 g, 14.4 g, and 36.0 g of NaCl were added

to the stock solutions to produce saturation levels of 10%, 20%, 30%, 40%, and 100% weight-per-volume (w/v) respectively. All samples were allowed to equilibrate for 10 minutes while being stirred at 750 rpm prior to extraction. The optimal temperature and extraction times previously determined were used throughout the remainder of the study.

Statistical Analysis

In order to determine the optimal range of each extraction parameter, the data was either visually examined, or a test for comparison was conducted. All sets of triplicate samples for the ranges under investigation were independently reviewed in a manner similar to that outlined for fiber optimization. RSDs were determined to exam reproducibility. ANOVA was conducted to compare the variation in peak areas in and among each point in the range of the parameter under investigation to identify similarities or differences. The residuals were examined for normality. To determine the optimal value in the range of points under investigation, a visual examination of the data could have been used. When determining an optimal extraction time, the optimal time may not represent the point at which the fiber was at equilibrium, but a point that was approaching equilibrium to ensure sufficient sensitivity for the method while reducing the total time of analysis. To verify visual analysis, Tukey's *Post Hoc* comparison and the Dunnett's t-test for comparing a treatment to a control were used. For Dunnett's t-test, each point in the range was compared to one point that was chosen as the control to determine whether they were statistically similar or different[19]. The last point in the range for time (100 minutes), the lowest temperature (30°C), and 0% saturation were used as controls. This test helped to determine at which specific points the values were statistically different. The point prior to this, or the point at which the highest mean peak area was produced, was chosen as the optimal value for the range under investigation.

Water Source Comparison - Fiber and Method Sensitivity

Conditions

The final step in this study was to determine the sensitivity of the selected fibers and method. Sensitivities were determined by spiking each water source with a series of stock solutions produced through serial dilution containing pure analytical standards of each pesticide at continuously lower known concentrations. The sensitivity of the fiber and method for each pesticide was defined as the lowest concentration that was detected and allowed for quantification of GC peak area responses. A qualified independent laboratory (Martel Laboratories, Baltimore, MD) determined the hardness, turbidity, suspended solids, total dissolved solids, and total organic carbon levels of each water source so that comparisons could be made to determine whether these characteristics interfered with the extraction of analytes.

Methods

Stock solutions of 50 mg/L and 100 mg/L of each pesticide were prepared in a similar manner as previous work in this study. The 100 mg/L stock was further diluted to 10 mg/L by adding 10 mL of stock to 90 mL of ultra-pure water. The 10 mg/L and 50 mg/L stocks were used to produce the initial dilution concentrations of 1.0 mg/L and 5.0 mg/L by adding 10 mL of each stock to 90 mL of the source water under investigation. Serial dilutions were continued until 100 mL stock solutions with concentrations of 5.0, 1.0, 0.5, 0.1, 0.05, 0.01, 0.005, and 0.001 mg/L were produced. If salting was required, the salt was not added to the diluted sample until the 10 mL needed for the next dilution had been drawn to ensure a uniform rate of salting across each serial dilution. The control (ultra-pure water) and raw surface water were not altered in any way. Modeled ground water was prepared in accordance with standards identified by the American Society for Testing and Materials (ASTM) by diluting 1.64 g sodium chloride (Fisher Scientific,

Suwanee, GA) and 1.48 g anhydrous sodium sulfate (Sigma-Aldrich, St. Louis, MO) to exactly 1.0 L with ultra-pure (HPLC-grade) water. One hundred (100) mL of this stock was then further diluted to a total volume of 1.0 L to produce modeled ground water[14,30]. A 1.0 ppm free available chlorine stock solution was prepared to simulate a post-production water source by dissolving 0.001 g of calcium hypochlorite in 1.0 L of ultra-pure water. The optimal matrix temperatures, extraction times, and ionic strengths were used for this final portion of the study.

Statistical Analysis

RSDs were determined to examine reproducibility at each concentration. Regression analysis was conducted to determine whether the known range of concentrations of each pesticide spiked into each water source produced a linear response. In order to determine the nature and strength of the relationship between the independent variable (concentration) and dependent variable (peak area), regression and correlation analysis was conducted[19]. This analysis was helpful to determine whether the peak areas produced from the fiber, analytical method, and equipment were a result of a sample's concentration, or due to some random error. The nature of the relationship was determined by the regression coefficient of determination (R-square), which explained how much of the variability in the peak area was due to the concentration of the sample. The correlation coefficient (R) was determined to examine the strength of the linear relationship between the two variables[19]. These values were used to describe the relationships between the two variables for each water source for both the laboratory and field analytical systems. The mean peak areas (GC response in area counts) were plotted on the abscissa as a function of each concentration of pesticide ordinate to observe whether a linear response occurred.

CHAPTER FOUR: RESULTS AND DATA ANALYSIS

SPME Fiber Selection/Optimization

Carbaryl

Table 4.1 shows the statistical analysis findings for each data set related to the seven fibers that were compared. The sample set for the 7 μm PDMS fiber was repeated three times and each time displayed very high variance (≥ 86.6%). The ANOVA F-statistic exceeded the critical F-value of 6.61 and all p-values from Tukey's *Post Hoc* comparison were significant ($p < 0.05$). The resulting F-statistic and p-values indicate the null hypothesis (all fibers are the same) should be rejected and conclude they are different. Therefore, the mean peak areas produced by each fiber are statistically different. The sample size used for this study was small; however, this sample size has not been discriminated against by peer reviewers of prominent scientific journals and is routinely used for this type of study. The 70 μm CW/DVB fiber exhibited low variance, excellent reproducibility, and produced the highest mean peak area and was chosen as the optimal fiber for immersion SPME of carbaryl.

Table 4.1 – SPME Fiber Selection/Optimization
GC-MS Extracted Ion Chromatogram Area for Carbaryl Peak
30 Minute Immersion Extraction, 50°C

SPME Fiber Selection/Optimization							
	7 PDMS	30 PDMS	100 PDMS	85 PA	65 PDMS/DVB	70 CW/DVB	75 CAR/PDMS
Mean	2,72,053	29,132,979	74,052,602	198,128,593	235,559,919	313,189,053	121,546,632
STD DEV	2,060,381	4,028,175	554,612	2,923,360	20,356,297	9,591,602	9,993,380
RSD (%)	86.6	13.83	0.75	1.48	8.64	3.06	8.22
ANOVA $F_{1,5}$ = 431.862							
p<0.000							

Tukey's *Post Hoc* Comparison with p-values reported							
	7 PDMS	30 PDMS	100 PDMS	85 PA	65 PDMS/DVB	70 CW/DVB	75 CAR/PDMS
7 PDMS	N/A	0.048	0.000	0.000	0.000	0.000	0.000
30 PDMS	0.048	N/A	0.001	0.000	0.000	0.000	0.000
100 PDMS	0.000	0.001	N/A	0.000	0.000	0.000	0.000
85 PA	0.000	0.000	0.000	N/A	0.004	0.000	0.000
65 PDMS/DVB	0.000	0.000	0.000	0.004	N/A	0.000	0.000
70 CW/DVB	0.000	0.000	0.000	0.000	0.000	N/A	0.000
75 CAR/PDMS	0.000	0.000	0.000	0.000	0.000	0.000	N/A
***Post Hoc* comparison**							
p<0.05 indicates a significant difference exists between fibers under comparison							
All fibers were found to be significantly different							

Lindane (γ-BHC)

Table 4.2 shows the statistical analysis values for each data set with group comparisons. The RSDs for all data sets indicate that similar reproducibility can be expected no matter which fiber is chosen for the extraction of lindane. Although the F-statistic exceeded the critical value of 6.61, most p-values for Tukey's post-hoc comparisons were not found to be significant ($p > 0.05$), indicating similarity in variance and peak areas across fibers. Table 4.3 shows the output of Tukey's *Post Hoc* comparison produced using SPSS software (SPSS Inc., Chicago, IL), which organizes fibers into subsets of statistically identical fibers. Three fibers were common across subsets; however, none produced the highest mean peak area of all fibers. The 30 μm PDMS fiber was statistically similar to the three that overlapped, produced the highest mean peak area,

had low variance, and is well suited to the extraction of non-polar substances, which make it appropriate for the extraction of lindane from water. Therefore, it was chosen as the optimal fiber for lindane and used throughout the remainder of the study.

Table 4.2 – SPME Fiber Selection/Optimization
GC-MS Extracted Ion Chromatogram Area for Lindane Peak
30 Minute Immersion Extraction, 50°C

SPME Fiber Selection/Optimization							
	7 PDMS	30 PDMS	100 PDMS	85 PA	65 PDMS/DVB	70 CW/DVB	75 CAR/PDMS
Mean	47,737,491	50,354,946	42,664,663	43,713,822	36,833,899	44,326,149	38,360,366
STD DEV	1,777,699	1,927,628	1,809,614	2,536,051	1,079,537	6,503,328	2,140,391
RSD (%)	3.72	3.83	4.24	5.8	2.93	14.67	5.58
ANOVA $F_{1,5}$ = 7.430							
p<0.001							
Tukey's *Post Hoc* Comparison with p-values reported							
	7 PDMS	30 PDMS	100 PDMS	85 PA	65 PDMS/DVB	70 CW/DVB	75 CAR/PDMS
7 PDMS	N/A	0.931	0.431	0.672	0.008	0.806	0.026
30 PDMS	0.931	N/A	0.087	0.175	0.001	0.256	0.004
100 PDMS	0.431	0.087	N/A	0.999	0.287	0.992	0.606
85 PA	0.672	0.175	0.999	N/A	0.150	1.000	0.374
65 PDMS/DVB	0.008	0.001	0.287	0.150	N/A	0.100	0.995
70 CW/DVB	0.806	0.256	0.992	1.000	0.100	N/A	0.265
75 CAR/PDMS	0.026	0.004	0.606	0.374	0.995	0.265	N/A
***Post Hoc* comparison**							
p<0.05 indicates a significant difference exists between fibers under comparison							
Two sets of five fibers were determined to be homogenous							

Table 4.3 – Tukey's HSD Multiple Comparison (with mean peak areas)

Fiber	Subset 1	Subset 2
65 um PDMS/DVB	36833899	
75 um CAR/PDMS	38360366	
100 um PDMS	42664663	42664663
85 um PA	43713822	43713822
70 um CW/DVB	44326149	44326149
7 um PDMS		47737491
30 um PDMS		50354946

Optimization of Extraction Parameters and Matrix Effects

Carbaryl

Extraction Temperature Optimization

Table 4.4 shows all statistical analysis values for each set of corresponding data with analysis of variance and treatment to control comparisons. No peaks for the pesticides of interest were detected from samples extracted at 100°C. Therefore, 100°C was not used for comparative evaluations. Greater variance in peak area was associated with the first and last temperatures in the range of temperatures that were investigated. The mean peak areas consistently decreased as temperature increased. The F-statistic exceeded the critical value of 6.61 indicating a significant finding that concludes that different temperatures generally give different responses, which is important although not statistically significant across all temperatures investigated. Dunnett's t-test supports this finding using the lowest temperature as the control. The 30°C extraction temperature produced the highest mean peak area, but was statistically identical to 40°C. Since the 40°C results were identical, and exhibited higher reproducibility, it was chosen as the optimal extraction temperature for carbaryl and used throughout the remainder of the study.

Table 4.4 – Optimal SPME Extraction Temperature
GC-MS Extracted Ion Chromatogram Area for Carbaryl Peak,
70 µm CW/DVB SPME Fiber, 30 Minute Immersion Extraction

Optimal SPME Extraction Temperature

	30° C	40° C	50° C	60° C	70° C	80° C	90° C
Mean	258,176,363	257,334,124	232,912,546	187,691,119	130,092,901	35,642,561	1,136,324
STD DEV	28,353,699	7,804,609	6,963,953	2,194,313	6,766,728	2,934,811	210,266
RSD (%)	10.98	3.03	2.99	1.17	5.2	8.23	18.5

ANOVA $F_{1,5}$ = 240.210
p<0.000

Tukey's *Post Hoc* Comparison with p-values reported

	30° C	40° C	50° C	60° C	70° C	80° C	90° C
30° C	N/A	1.000	0.190	0.000	0.000	0.000	0.000
40° C	1.000	N/A	0.218	0.000	0.000	0.000	0.000
50° C	0.190	0.218	N/A	0.005	0.000	0.000	0.000
60° C	0.000	0.000	0.005	N/A	0.001	0.000	0.000
70° C	0.000	0.000	0.000	0.001	N/A	0.000	0.000
80° C	0.000	0.000	0.000	0.000	0.000	N/A	0.037
90° C	0.000	0.000	0.000	0.000	0.000	0.037	N/A

***Post Hoc* comparison**
p<0.05 indicates a significant difference exists between temperatures under comparison
Three temperatures were determined to be homogenous

Dunnett's Procedure for Comparing a Treatment to a Control

	40° C	50° C	60° C	70° C	80° C	90° C
30° C	1.000	0.085	0.000	0.000	0.000	0.000

Dunnett-t (2-sided)
p<0.05 indicates a significant difference exists between temperatures under comparison
Compared to 30° C, 40°C and 50°C are homogenous

Extraction Time Optimization

Table 4.5 shows the results of the statistical analysis of each set of corresponding data with comparative statistics. The RSDs associated with each extraction time were low across the sets indicating excellent reproducibility at each point. The auto-sampler's software limited extraction time to no more than 100 minutes; therefore, longer extractions were not performed. Previous research indicated that some fibers had not reached equilibrium for certain compounds prior to this point, but the results of this study were conclusive that equilibrium was likely reached prior to 100 minutes. The F-statistic exceeded the critical value of 5.99 for a 5% significance level indicating a significant finding and that the null hypothesis (all temperatures are similar in

regards to the dependent variable) should be rejected. Most p-values from Tukey's *Post Hoc* comparison support this conclusion. From p-value comparison, and Dunnett's t-test, it appears equilibrium occurred, or was approached at 15 minutes. For a rapid field screening method, a time that quickly yields sufficient sensitivity and precision and is approaching equilibrium is desirable. Since 15 minutes produced the highest mean peak area, exhibited good reproducibility, and is a relatively short extraction time, it was chosen as the optimal extraction time for carbaryl and used for the remainder of the study.

Table 4.5 – Optimal SPME Extraction Time
GC-MS Extracted Ion Chromatogram Area for Carbaryl Peak
70 µm CW/DVB SPME Fiber, 40°C

Optimal SPME Extraction Time

	1 min	5 min	15 min	30 min	45 min	60 min	90 min	100 min
Mean	131,992,212	202,598,144	247,669,995	231,509,161	224,809,827	208,948,665	226,694,468	204,330,573
STD DEV	12,868,769	2,747,036	10,099,862	8,793,971	13,044,637	12,960,962	6,404,402	8,970,118
RSD (%)	9.75	1.36	4.08	3.8	5.8	6.2	2.83	4.39

ANOVA $F_{1,6} = 36.107$
p<0.000

Tukey's *Post Hoc* Comparison with p-values reported

	1 min	5 min	15 min	30 min	45 min	60 min	90 min	100 min
1 min	N/A	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5 min	0.000	N/A	0.001	0.045	0.192	0.992	0.131	1.000
15 min	0.000	0.001	N/A	0.531	0.169	0.005	0.243	0.002
30 min	0.000	0.045	0.531	N/A	0.990	0.179	0.999	0.067
45 min	0.000	0.192	0.169	0.990	N/A	0.553	1.000	0.267
60 min	0.000	0.992	0.005	0.179	0.553	N/A	0.423	0.999
90 min	0.000	0.131	0.243	0.999	1.000	0.423	N/A	0.186
100 min	0.000	1.000	0.002	0.067	0.267	0.999	0.186	N/A

***Post Hoc* comparison**
p<0.05 indicates a significant difference exists between times under comparison
Multiple times were considered homogenous

Dunnett's Procedure for Comparing a Treatment to a Control

	1 min	5 min	15 min	30 min	45 min	60 min	90 min
100 min	0.000	1.000	0.000	0.024	0.113	0.990	0.074

Dunnett-t (2-sided)
p<0.05 indicates a significant difference exists between times under comparison
Fiber is approaching equilibrium at 15 minutes

Ionic Strength (Salting) Optimization

Table 4.6 shows the GC-MS peak areas achieved at each ionic strength and the comparative statistics. The RSDs for all saturation levels were higher than the RSD achieved with no saturation. The higher variance for the sets that were salted indicates the extraction of carbaryl is less reproducible when the sample ionic strength is adjusted with NaCl. The F-statistic exceeded the critical value of 7.71 at the 5% significance level, which was a significant finding and demonstrated the ionic strengths under comparison were statistically different. Most p-values of Tukey's *Post Hoc* comparisons support this finding and it can be concluded from this information that 0% and 100% saturation were statistically different than all other levels. The mean peak areas decreased rapidly with saturation. A visual observation of the fiber following each set of runs indicated excessive fouling of the fiber coating with salt even at lower saturation levels. The 0% saturation level was chosen to be the optimum ionic strength for the extraction of carbaryl. Salting had no positive effect and 0% saturation produced the highest mean peak area, showed excellent reproducibility, and would not subject the fiber to fouling that would reduce its longevity.

Table 4.6 – Optimal SPME Ionic Strength
GC-MS Extracted Ion Chromatogram Area for Carbaryl Peak
70 µm CW/DVB SPME Fiber, 40°C, and 15 Minute Immersion Extraction

Optimal SPME Ionic Strength						
	0%	10%	20%	30%	40%	100%
Mean	251,381,638	69,887,004	43,385,244	57,311,401	76,038,426	144,736,340
STD DEV	4,874,692	33,831,093	11,062,055	11,610,779	6,367,876	17,645,759
RSD (%)	1.94	48.41	25.5	20.26	8.37	12.19
ANOVA $F_{1,4}$ = 63.035						
p<0.000						

Tukey's *Post Hoc* Comparison with p-values reported						
	0%	10%	20%	30%	40%	100%
0%	N/A	0.000	0.000	0.000	0.000	0.000
10%	0.000	N/A	0.454	0.941	0.997	0.002
20%	0.000	0.454	N/A	0.912	0.257	0.000
30%	0.000	0.941	0.912	N/A	0.763	0.000
40%	0.000	0.997	0.257	0.763	N/A	0.004
100%	0.000	0.002	0.000	0.000	0.004	N/A

***Post Hoc* comparison**
p<0.05 indicates a significant difference exists between ionic strengths under comparison
Mean peak area significantly larger for 0% saturated

Dunnett's Procedure for Comparing a Treatment to a Control					
	10%	20%	30%	40%	100%
0%	0.000	0.000	0.000	0.000	0.000

Dunnett-t (2-sided)
p<0.05 indicates a significant difference exists between ionic strengths under comparison
0% saturation significantly different than all other saturation levels

Lindane (γ-BHC)

Extraction Temperature Optimization

Table 4.7 shows the GC-MS peak areas achieved for each extraction temperature and analysis of variance and treatment to control comparisons. The RSDs varied across groups and displayed the best reproducibility at 60, 70, 90, and 100°C. The F-statistic was lower than the critical value of 5.99 at a 95% confidence interval indicating a finding that was not significant. Therefore, it could be concluded that there was similarity between extraction temperatures. However, the p-

value for the same test contradicts this since it was below 0.05. Most p-values across sets from Tukey's Post Hoc analysis were not significant ($p > 0.05$) and support the F-statistic, which concludes the null hypothesis should be rejected and the mean peak areas produced by different extraction temperatures are similar. The p-value for the 40°C and 70°C pair-wise comparison was the only comparison that did not exceed the critical level for the 95% confidence interval. Dunnett's test concludes that all extraction temperatures are similar when compared to the control (lowest temperature). Therefore, since the mean peak area produced during the extractions at 40°C was the highest, this temperature will be used as the optimal temperature.

Table 4.7 – Optimal SPME Extraction Temperature
GC-MS Extracted Ion Chromatogram Area for Lindane Peak
30 µm PDMS SPME Fiber, 30 Minute Immersion Extraction

Optimal SPME Extraction Temperature

	30° C	40° C	50° C	60° C	70° C	80° C	90° C	100° C
Mean	67,724,450	72,592,488	62,608,296	51,101,025	47,485,539	56,815,533	58,316,686	51,770,396
STD DEV	8,302,918	12,860,459	12,095,848	1,623,993	1,761,001	13,093,769	421,034	697,756
RSD (%)	12.26	17.72	19.32	3.18	3.71	23.05	0.72	1.35

ANOVA $F_{1,6} = 3.222$
$p<0.025$

Tukey's *Post Hoc* Comparison with p-values reported

	30° C	40° C	50° C	60° C	70° C	80° C	90° C	100° C
30° C	N/A	0.995	0.994	0.288	0.123	0.745	0.854	0.332
40° C	0.995	N/A	0.815	0.089	0.033	0.344	0.458	0.106
50° C	0.994	0.815	N/A	0.695	0.392	0.987	0.998	0.750
60° C	0.288	0.089	0.695	N/A	0.999	0.988	0.957	1.000
70° C	0.123	0.033	0.392	0.999	N/A	0.859	0.751	0.998
80° C	0.745	0.344	0.987	0.988	0.859	N/A	1.000	0.994
90° C	0.854	0.458	0.998	0.957	0.751	1.000	N/A	0.974
100° C	0.332	0.106	0.750	1.000	0.998	0.994	0.974	N/A

***Post Hoc* comparison**
$p<0.05$ indicates a significant difference exists between temperatures under comparison
Two subsets of 7 temperatures each were found to be homogenous

Dunnett's Procedure for Comparing a Treatment to a Control

	40° C	50° C	60° C	70° C	80° C	90° C	100° C
30° C	0.964	0.955	0.124	0.046	0.468	0.610	0.148

Dunnett-t (2-sided)
$p<0.05$ indicates a significant difference exists between temperatures under comparison
Compared to 30° C, all other temperatures are homogenous except for 70°C

Extraction Time Optimization

Table 4.8 shows the GC-MS peak areas achieved for each extraction time with comparative statistics. RSDs were high across all extraction time sets with two exceptions, 45 and 90 minutes. The higher RSDs observed may be due to the use of a lower stock solution concentration (15 mg/L) for lindane. A concentration of 100 mg/L was not used due to the compound's low water solubility and its tendency to produce larger masses of undissolved solids in solution. The F-statistic was lower than the critical value of 5.99 for a 5% significance level along with the test's p-value (0.155), which indicates a finding that is not significant. A conclusion was made that the general level of variance in peak areas across all extraction times was statistically similar. As with variance, it was concluded that the mean peak areas produced for all extraction times were statistically similar. Therefore, the one-minute extraction time should provide statistically similar results as the 100-minute extraction time. However, this pesticide is likely to be found in trace quantities in environmental waters and a longer sample time may be warranted to ensure enough time for the compound to partition to a quantifiable point. At 15 minutes, the greatest mean peak area is produced. Therefore, 15 minutes will be used as the optimal extraction time for lindane.

Table 4.8 – Optimal SPME Extraction Time
GC-MS Extracted Ion Chromatogram Area for Lindane Peak
30 µm PDMS SPME Fiber, 40°C

Optimal SPME Extraction Time

	1 min	5 min	15 min	30 min	45 min	60 min	90 min	100 min
Mean	67,664,425	57,382,559	75,101,267	56,191,933	49,359,460	60,756,429	67,318,094	61,904,346
STD DEV	16,840,915	7,702,507	12,565,449	5,641,339	539,899	11,886,411	1,227,304	13,280,894
RSD (%)	24.89	13.42	16.73	10.04	1.09	19.56	1.82	21.45

ANOVA $F_{1,6}$ = 1.804
p<0.155

Tukey's *Post Hoc* Comparison with p-values reported

	1 min	5 min	15 min	30 min	45 min	60 min	90 min	100 min
1 min	N/A	0.914	0.984	0.861	0.415	0.989	1.000	0.996
5 min	0.914	N/A	0.453	1.000	0.975	1.000	0.927	0.999
15 min	0.984	0.453	N/A	0.378	0.105	0.686	0.979	0.763
30 min	0.861	1.000	0.378	N/A	0.990	0.999	0.878	0.997
45 min	0.415	0.975	0.105	0.990	N/A	0.865	0.437	0.803
60 min	0.989	1.000	0.686	0.999	0.865	N/A	0.992	1.000
90 min	1.000	0.927	0.979	0.878	0.437	0.992	N/A	0.998
100 min	0.996	0.999	0.763	0.997	0.803	1.000	0.998	N/A

***Post Hoc* comparison**
p<0.05 indicates a significant difference exists between times under comparison
All extraction times are statistically homogenous

Dunnett's Procedure for Comparing a Treatment to a Control

	1 min	5 min	15 min	30 min	45 min	60 min	90 min
100 min	0.971	0.992	0.488	0.972	0.537	1.000	0.979

Dunnett-t (2-sided)
p<0.05 indicates a significant difference exists between times under comparison
Fiber appears to approach equilibrium at 1 minute

Ionic Strength (Salting) Optimization

Table 4.9 shows the GC-MS peak areas achieved for each ionic strength with comparative statistics. The variance produced as a result of salting was higher for 0% and 20% saturation. The F-statistic was lower than the critical value of 7.71 indicating similarity across saturation levels. The pair-wise comparisons of Tukey's analysis indicate that most saturation levels are statistically similar. However, Dunnett's treatment to control comparison indicates that most saturation levels are statistically different. The 20% saturation level produced the highest mean

peak area, but was not statistically different from 10% saturation. A visual observation of the fiber coating following each set of runs indicated excessive fouling of the fiber coating for all levels of salting. However, since some level of saturation did seem to drastically improve the mean peak areas produced, the 0% saturation level may not be the optimal range under this system. Despite producing the highest mean peak areas, the 20% saturation level may have a disadvantage due to the potential to foul the fiber quicker. Therefore, the 10% saturation level was chosen as the optimal point since the mean peak area produced is nearly identical to the 20% mean and the potential to foul the fiber coating can be decreased by using a lower w/v saturation level.

Table 4.9 – Optimal SPME Ionic Strength
GC-MS Extracted Ion Chromatogram Area for Lindane Peak
30 µm PDMS SPME Fiber, 40°C, and 15 Minute Immersion Extraction

Optimal SPME Ionic Strength						
	0%	10%	20%	30%	40%	100%
Mean	47,187,616	74,982,910	80,584,142	73,469,183	74,975,872	72,025,814
STD DEV	23,155,478	1,559,729	10,527,653	1,768,035	1,483,759	2,069,923
RSD (%)	49.07	2.08	13.06	2.41	1.98	2.87
ANOVA $F_{1,4} = 3.804$						
p<0.027						

Tukey's *Post Hoc* Comparison with p-values reported						
	0%	10%	20%	30%	40%	100%
0%	N/A	0.060	0.020	0.081	0.060	0.106
10%	0.060	N/A	0.984	1.000	1.000	0.999
20%	0.020	0.984	N/A	0.956	0.984	0.909
30%	0.081	1.000	0.956	N/A	1.000	1.000
40%	0.060	1.000	0.984	1.000	N/A	0.999
100%	0.106	0.999	0.909	1.000	0.999	N/A
***Post Hoc* comparison**						
p<0.05 indicates a significant difference exists between ionic strengths under comparison						
Mean peak area homogenous expect for 0% and 20% saturation						

Dunnett's Procedure for Comparing a Treatment to a Control					
	10%	20%	30%	40%	100%
0%	0.027	0.009	0.037	0.027	0.050
Dunnett-t (2-sided)					
p<0.05 indicates a significant difference exists between ionic strengths under comparison					
All levels statistically different from control expect for 100% saturation					

Water Source Comparison – Fiber and Method Sensitivity

Lab-based GC-MS (Agilent 6890)

Carbaryl

Table 4.10 shows data from sampling and analysis for carbaryl in ultra-pure water (control) samples with concentrations ranging from 0.01 to 5.0 mg/L. Extracted ion chromatograms were produced with Chemstation software to obtain GC peak areas for each sample by doing extraction ion searches for carbaryl's principal ions (144, 115, and 127 *m/z*). The peak areas listed in each table are for the 144 *m/z* peaks only, which is the most predominant peak for this compound. The peak areas obtained from each pesticides predominant ion peak were used for analysis and comparison for each water source and for both analytical instruments. Carbaryl was detected and peak areas quantified down to a concentration of 0.1 mg/L, which is five-times lower than the MEG for this chemical. Signal-to-noise-ratios were well above 3:1 at all quantifiable and detectable concentrations. Peak areas were detectable, but not quantifiable, at 0.05 mg/L, or ten-times lower than the MEG. Consistent, low variance within sample sets down to 0.1 mg/L indicates the selected fiber and analytical method were capable of consistently reproducing similar results. Figure 4.1 shows the GC-MS mean peak area responses for known concentrations spiked into the control water. The regression statistics R-square (R^2) and R demonstrate the nature and strength of the relationship respectively between the concentration of the samples and the peak areas produced. The regression coefficient R^2 was 0.985365, which indicates that 98.54% of the total variance was explained as a result of a sample's concentration versus due to random error. The strength of this relationship was shown with the regression coefficient R (0.992656), which indicates a 99.27% correlation between the sample concentrations and peak areas produced.

Table 4.10 – Sensitivity of Fiber and Method for Carbaryl in Control 70 µm CW/DVB SPME Fiber, 40°C, and 15 Minute Immersion Extraction

	Control Serial Dilutions (mg/L)					
Sample #	0.01	0.05	0.1	0.5	1.0	5.0
1	NDP	PD/NQ	125,623	1,439,222	4,103,345	51,363,685
2	NDP	PD/NQ	131,922	1,496,614	4,170,961	52,644,767
3	NDP	PD/NQ	119,049	1,441,917	3,375,050	46,058,791
Mean	0	0	125,531	1,459,251	3,883,119	50,022,414
STD DEV	0	0	6,437	32,385	441,297	3,491,851
RSD (%)	0	0	5.13	2.22	11.36	6.98

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.1 – Detection of Carbaryl in Control with 70 µm CW/DVB Fiber

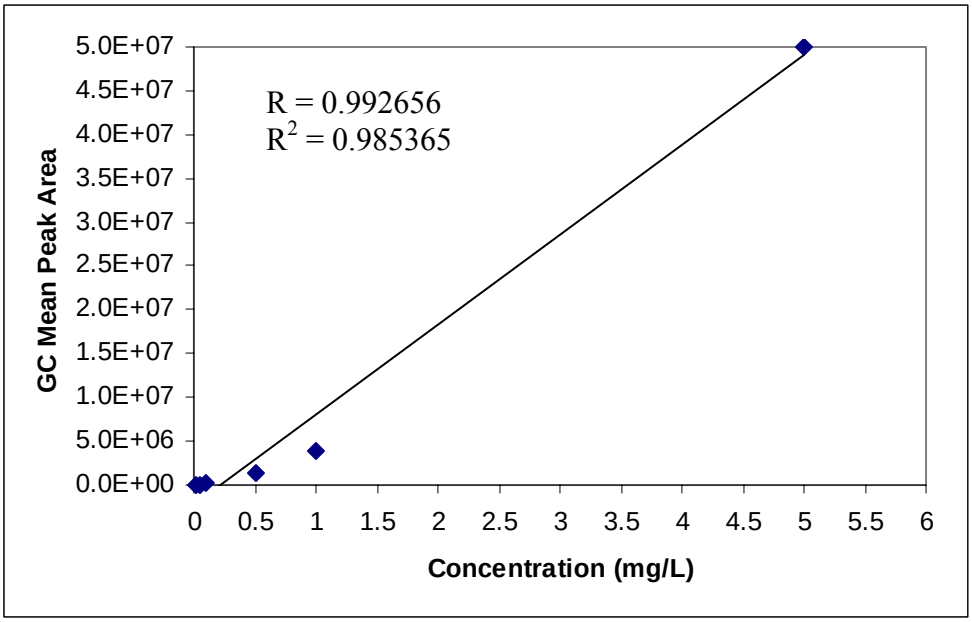


Table 4.11 shows data from sampling and analysis with modeled ground water. RSDs were higher in those sets corresponding to the lower concentrations with the highest variance seen at 0.5 mg/L. Carbaryl was detected and quantified down to a concentration of 0.05 mg/L, or ten-times lower than the lowest MEG. Peak areas were not detectable below this concentration. Figure 4.2 shows good linearity ($R^2 = 0.985863$, $R = 0.992907$) indicating a strong correlation between concentration and peak areas despite some fluctuation in RSDs as sample concentrations decreased. During method optimization, it was determined that no salting provided the best results for SPME with the 70 μm CW/DVB fiber for this compound. The natural salts in the modeled ground water may have improved the extraction efficiency of the fiber and method as the sensitivity was improved and the compound was detected and quantified down to 0.05 mg/L from 0.1 mg/L, which was determined for the control. A salting level somewhere between 0% and 10% saturation (w/v) may be ideal and further research in this area is indicated to fully optimize the procedure.

Table 4.11 – Sensitivity of Fiber and Method for Carbaryl in Modeled Ground Water 70 μm CW/DVB SPME Fiber, 40°C, and 15 Minute Immersion Extraction

	Modeled Ground Water Serial Dilutions (mg/L)					
Sample #	0.01	0.05	0.1	0.5	1.0	5.0
1	NDP	38,093	81,736	1,120,164	1,798,428	24,737,964
2	NDP	33,062	65,821	861,869	1,766,759	23,635,881
3	NDP	41,073	75,448	754,282	1,938,622	22,210,679
Mean	0	37,409	74,335	912,105	1,834,603	23,525,175
STD DEV	0	4,049	8,016	188,043	91,464	1,271,762
RSD (%)	0	10.82	10.78	20.62	4.99	5.41

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.2 – Detection of Carbaryl in Modeled Ground Water with 70 µm CW/DVB Fiber

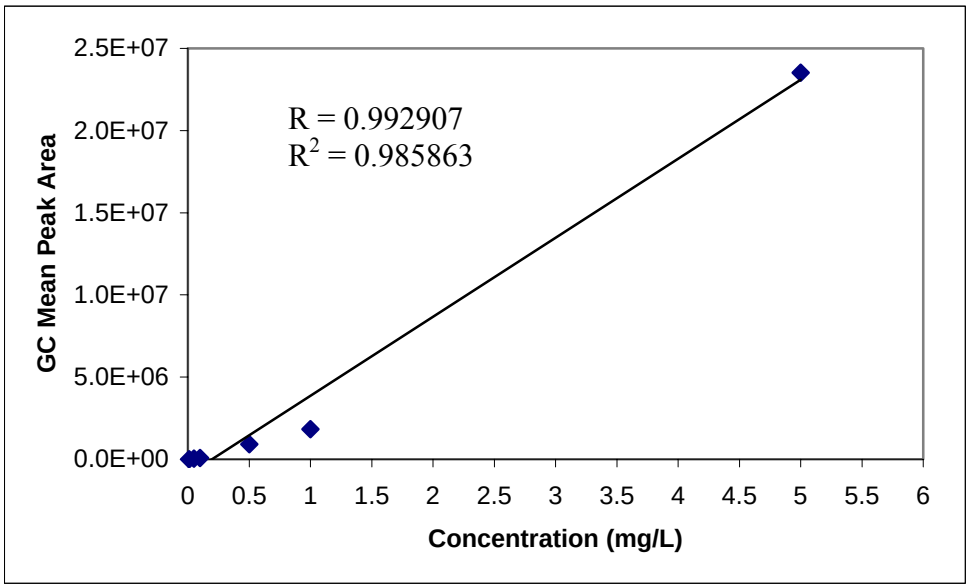


Table 4.12 shows data from sampling and analysis using simulated post-production water (1.0 ppm free available chlorine residual). RSDs were low across the range of concentrations. Carbaryl was detected and quantified in simulated post-production water down to a concentration of 0.01 mg/L, or 50 times lower than the lowest MEG. Peaks were not detectable below this concentration. The presence of calcium hypochlorite appears to have improved the sensitivity of the method by making carbaryl less water soluble and more able to partition into the fiber's coating. Figure 4.3 shows very good linearity (R^2 = 0.993874, R = 0.996932) indicating a strong correlation between concentration and peak areas for extraction from this source water.

Table 4.12 – Sensitivity of Fiber and Method for Carbaryl in Simulated Post-Production Water 70 µm CW/DVB SPME Fiber, 40°C, and 15 Minute Immersion Extraction

	Simulated Post Production Serial Dilutions (mg/L)					
Sample #	0.01	0.05	0.1	0.5	1.0	5.0
1	16,114	85,764	236,991	1,587,075	4,029,613	31,551,986
2	16,460	97,752	191,127	1,773,052	3,811,319	32,633,930
3	16,528	84,862	207,833	1,877,922	3,500,738	29,943,289
Mean	16,367	89,459	211,984	1,746,016	3,780,557	31,376,402
STD DEV	222	7,196	23,212	147,296	265,776	1,353,887
RSD (%)	1.36	8.04	10.95	8.44	7.03	4.31

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.3 – Detection of Carbaryl in Simulated Post Production Water with 70 µm CW/DVB Fiber

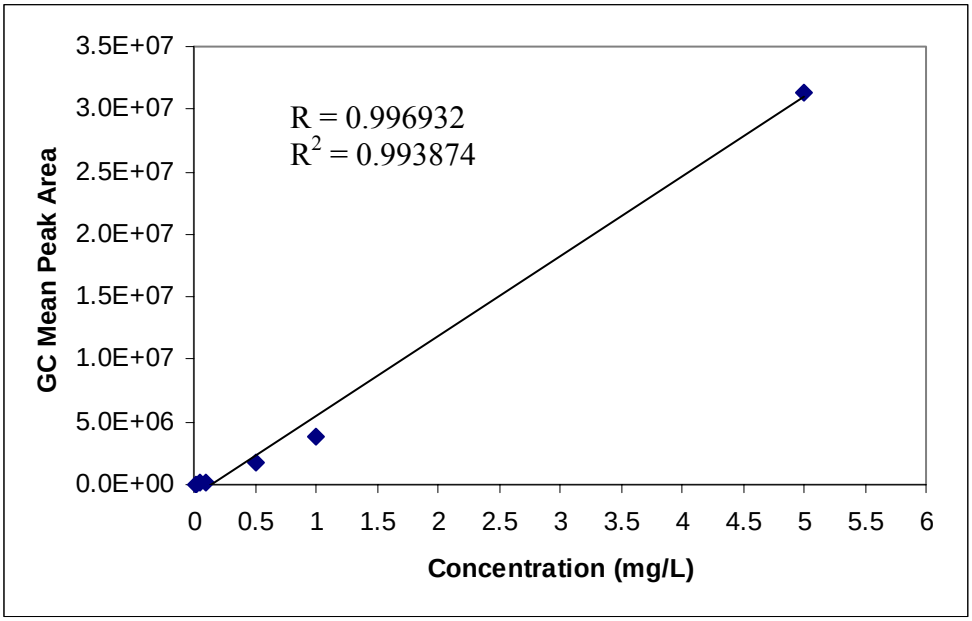


Table 4.13 shows data from sampling and analysis using raw surface water from a local pond in the Bethesda, MD area. RSDs varied across sets and the highest RSD was associated with the

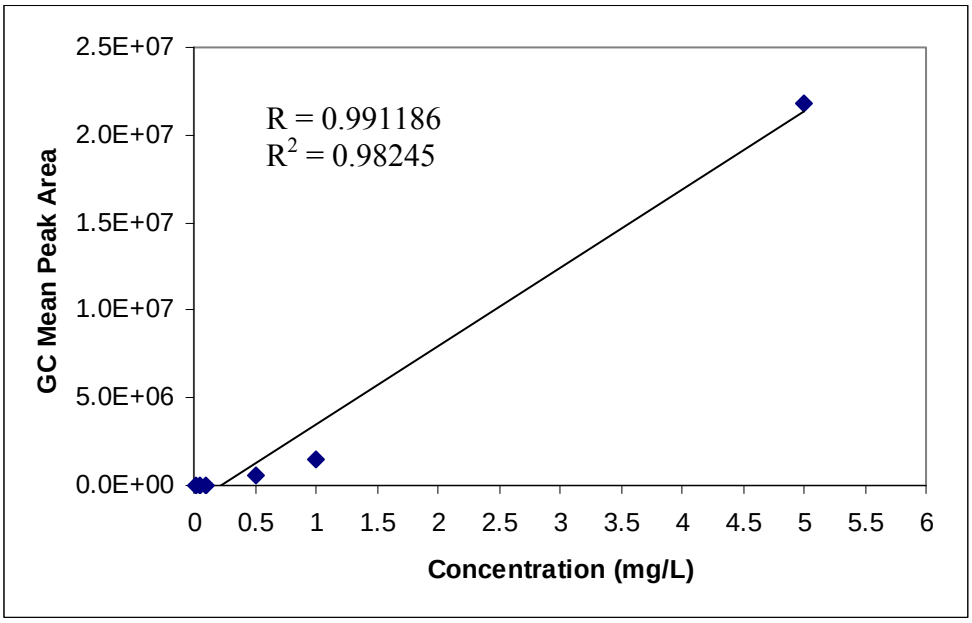
lowest concentration quantified. Carbaryl was detected and quantified down to a concentration of 0.05 mg/L in raw surface water. No peaks were detectable below this concentration. Figure 4.4 lists regression coefficients ($R^2 = 0.98245$, $R = 0.991186$) indicating a strong correlation between peak areas and pesticide concentration. The presence of a high level of suspended solids in the raw water may have contributed to the detection and quantification at a lower level than with the control. However, suspended solids and pollutants in raw water sources could potentially interfere with SPME when the target compounds are at low concentrations.

Table 4.13 – Sensitivity of Fiber and Method for Carbaryl in Raw Surface Water 70 µm CW/DVB SPME Fiber, 40°C, and 15 Minute Immersion Extraction

	Raw Surface Water Serial Dilutions (mg/L)					
Sample #	0.01	0.05	0.1	0.5	1.0	5.0
1	NDP	31,655	53,771	554,863	1,667,259	22,542,460
2	NDP	26,193	47,676	636,441	1,354,629	21,889,301
3	NDP	44,486	44,486	531,611	1,280,570	20,979,434
Mean	0	34,111	48,644	574,305	1,434,153	21,803,732
STD DEV	0	9,391	4,718	55,053	205,244	785,019
RSD (%)	0	27.53	9.7	9.59	14.31	3.6

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.4 – Detection of Carbaryl in Raw Surface Water with 70 µm CW/DVB Fiber



Lindane

Table 4.14 shows data from sampling and analysis with ultra-pure water. Extracted ion chromatograms were produced with Chemstation software to obtain GC peak areas for each sample by doing extraction ion searches of lindane's principal ions (181, 183, and 219 *m/z*). The peak areas listed are for the 181 *m/z* peaks only, which is the most predominant peak for this compound. The RSDs were low for data sets down to 0.05 mg/L, but significantly higher at 0.001 and 0.005 mg/L. The higher variance for the two lowest concentrations may have been the result of insufficient mixing of stock solutions. Since lindane is not very water soluble, a large mass of the compound may have been present in one of the three samples for these two concentrations resulting in significantly higher peak areas. Lindane was detected and quantified in the control water down to 0.005 mg/L, which is 40 times lower than the lowest MEG of 0.2

mg/L. Peaks were detectable at 0.001 mg/L, which was 200 times lower than the MEG. Due to lindane's low water solubility, it was expected that SPME with GC-MS analysis would be a capable system to detect and quantify the presence of lindane in water at very low concentrations. Other researchers were able to detect lindane in tap water at the µg/L and ng/L levels using GC with electron capture detectors (ECD)[19,31]. One of the conditions of the science and technical objective was that the equipment should be able to detect unknowns in a mixture. However, the ECD does not provide mass spectral information and is therefore of limited value in identification of unknowns. Figure 4.5 shows good linearity ($R^2 = 0.928396$, $R = 0.963533$) indicating a strong correlation between sample concentration and the lindane peak areas produced. The regression coefficients were not as high as those seen with carbaryl, which may be due to the nature of the compound and its affinity to partition to the chosen SPME fiber using this method. The same stock solution and methods were used for carbaryl and produced stronger regression coefficients. Therefore, the lower regression coefficients are likely due to the nature of the compound, or the chosen fiber, since all other parameters were comparable. However, the fiber and analytical method did prove to be a very capable detection method despite not producing optimal reproducibility of quantified data.

Table 4.14 – Sensitivity of Fiber and Method for Lindane in Control 30 µm PDMS Fiber, 40°C, and 15 Minute Immersion Extraction

	Control Serial Dilutions (mg/L)							
Sample #	0.001	0.005	0.01	0.05	0.1	0.5	1.0	5.0
1	PD/NQ	33,081	183,820	421,901	1,686,371	5,945,675	24,003,848	51,141,210
2	PD/NQ	161,00	125,728	395,272	1,611,231	5,330,701	23,495,068	50,715,713
3	PD/NQ	34,643	95,462	407,410	1,602,882	5,392,002	24,192,616	48,985,962
Mean		76,341	135,003	408,194	1,633,495	5,556,126	23,897,177	50,280,962
STD DEV		73,581	44,903	13,332	45,982	338,749	360,801	1,141,503
RSD (%)		96.38	33.26	3.27	2.81	6.1	1.51	2.27

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.5 – Detection of Lindane in Control with 30 µm PDMS Fiber

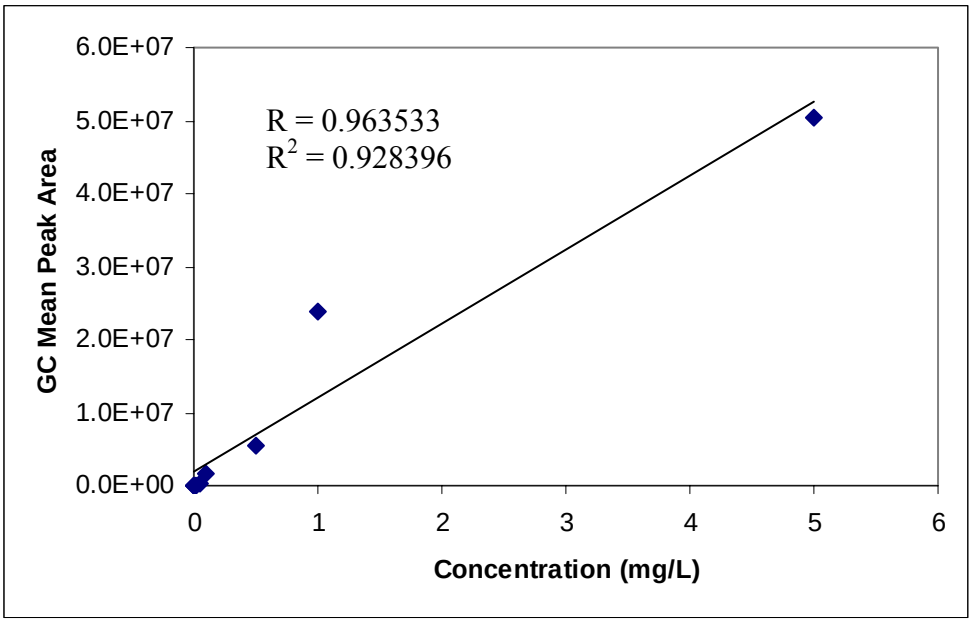


Table 4.15 shows data from sampling and analysis with modeled ground water. RSDs tended to be marginally higher at lower concentrations. Lindane was detected and quantified to a concentration of 0.005 mg/L. One of three samples was quantifiable at 0.001 mg/L, which indicates the method is more sensitive using this water source. During method optimization, 10% and 20% saturation were statistically equal; however, the mean peak area for 20% was higher. 10% saturation (w/v) was chosen as the optimal salting level since it produced statistically similar mean peak areas and did not foul the fiber as quickly. The additional salts present in the modeled ground water may have contributed to the ability of the method and system to quantify the one sample at a concentration five-times lower than was seen with the control. Figure 4.6 shows good linearity (R^2 = 0.961781, R = 0.980705) indicating a strong correlation between sample concentration and peak area.

Table 4.15 – Sensitivity of Fiber and Method for Lindane in Modeled Ground Water 30 µm PDMS Fiber, 40°C, and 15 Minute Immersion Extraction

	Modeled Ground Water Serial Dilutions (mg/L)							
Sample #	0.001	0.005	0.01	0.05	0.1	0.5	1.0	5.0
1	PD/NQ	43,050	106,305	360,198	1,106,651	4,495,351	16,193,730	39,548,639
2	PD/NQ	57,938	94,678	403,526	1,011,076	4,875,381	15,736,923	40,529,077
3	13,902	43,128	98,913	466,025	951,636	4,747,018	15,483,580	39,878,335
Mean	0	48,039	99,965	409,916	1,023,121	4,705,917	15,804,744	39,985,350
STD DEV	0	8,573	5,884	53,202	78,206	193,320	359,900	498,903
RSD (%)	0	17.85	5.89	12.98	7.64	4.11	2.28	1.25

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.6 – Detection of Lindane in Modeled Ground Water with 30 µm PDMS Fiber

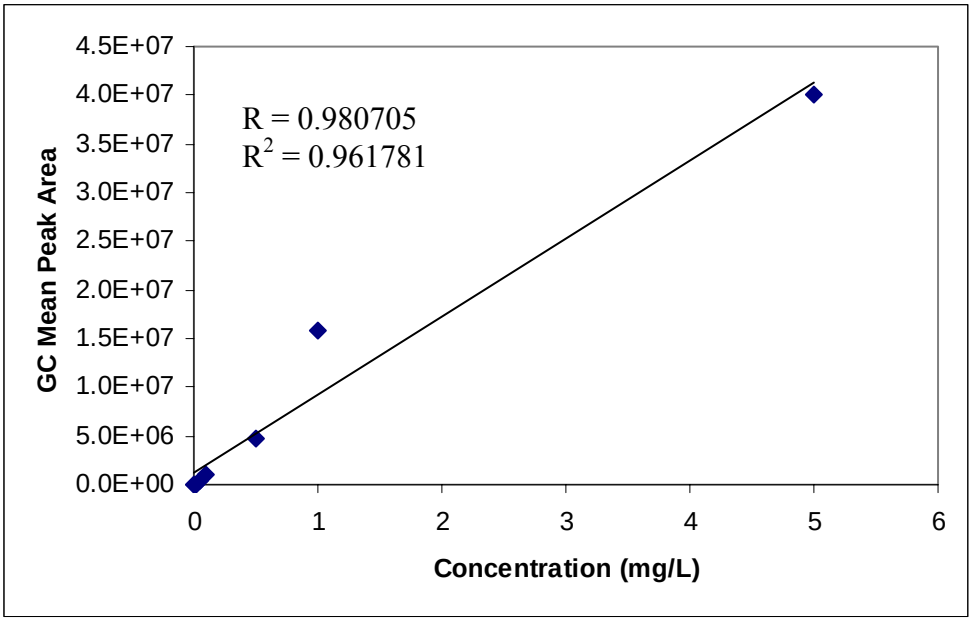


Table 4.16 shows data from sampling and analysis using simulated post-production water. Lindane was detected and quantified to a concentration of 0.005 mg/L. Two of three samples at 0.001 mg/L were quantifiable. Extreme variance was observed at 0.005 mg/L (RSD = 119.62).

Two of the three samples were nearly identical at this concentration; however, one sample was extremely high compared to the other two. The higher peak area for the one sample was likely due to the presence of a large mass of the compound in that sample vial, which resulted in a high level of variance for the entire set. Figure 4.7 shows good linearity ($R^2 = 0.919506$, $R = 0.958909$); however, the nature and strength of the relationship of the two variables was likely reduced due to the extreme variance observed with the final dilution set and the mean peak area from the 1.0 mg/L standard. The presence of calcium hypochlorite seemed to enhance the sensitivity of the method by reducing lindane's water solubility.

Table 4.16 – Sensitivity of Fiber and Method for Lindane in Simulated Post-Production Water 30 µm PDMS Fiber, 40°C, and 15 Minute Immersion Extraction

	Simulated Post Production Water Serial Dilutions (mg/L)							
Sample #	0.001	0.005	0.01	0.05	0.1	0.5	1.0	5.0
1	PD/NQ	22,809	71,002	188,356	854,235	2,018,836	16,525,504	42,972,133
2	14,652	170,303	77,945	185,224	884,310	2,066,207	20,173,988	31,596,336
3	15,473	21,444	75,680	167,215	918,229	2,078,453	15,215,604	32,841,922
Mean	0	71,519	74,876	180,265	885,591	2,054,499	17,305,032	35,803,464
STD DEV	0	85,552	3,541	11,410	32,016	31,486	2,569,463	6,239,410
RSD (%)	0	119.62	4.73	6.33	3.62	1.53	14.85	17.43

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.7 – Detection of Lindane in Simulated Post Production Water with 30 µm PDMS Fiber

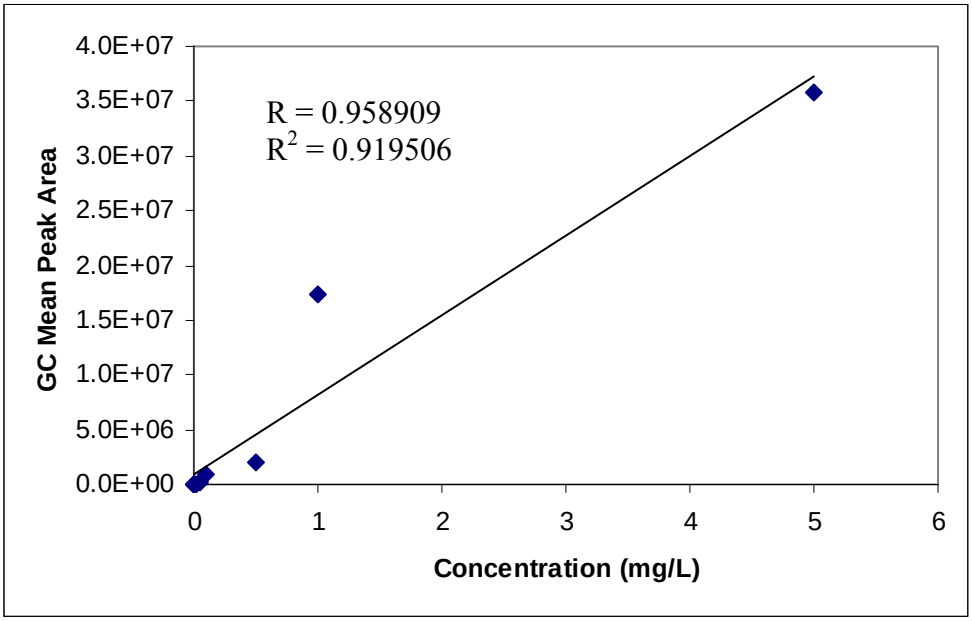


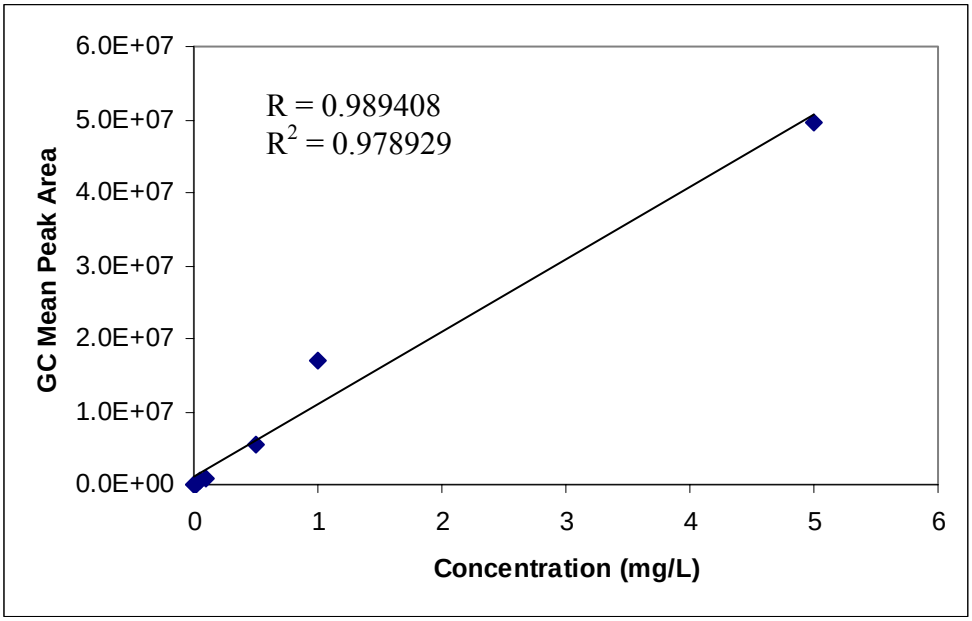
Table 4.17 shows data from sampling and analysis using raw surface water. Lindane was detected and quantified down to a concentration of 0.005 mg/L. Peaks were detected, but not quantifiable, below this level. The method and analytical system show excellent reproducibility for this compound at all concentrations sampled. The presence of a high level of suspended solids in this water source may have contributed to the detection and quantification at a lower level than the control. Figure 4.8 shows good linearity ($R^2 = 0.978929$, $R = 0.989408$), which indicates a strong correlation between sample concentration and peak areas.

Table 4.17 – Sensitivity of Fiber and Method for Lindane in Raw Surface Water 30 µm PDMS Fiber, 40°C, and 15 Minute Immersion Extraction

	Raw Surface Water Serial Dilutions (mg/L)							
Sample #	0.001	0.005	0.01	0.05	0.1	0.5	1.0	5.0
1	PD/NQ	53,309	90,355	481,653	938,693	5,315,640	17,158,520	49,852,714
2	PD/NQ	52,227	79,432	439,249	905,117	4,983,684	16,944,676	48,664,006
3	PD/NQ	49,953	95,340	453,715	886,477	6,011,868	16,773,023	50,049,848
Mean	0	51,830	88,376	458,206	910,096	5,437,064	16,958,740	49,522,189
STD DEV	0	1,713	8,137	21,556	26,462	524,737	193,133	749,716
RSD (%)	0	3.3	9.21	4.7	2.91	9.65	1.14	1.51

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.8 – Detection of Lindane in Raw Surface Water with 30 µm PDMS Fiber



Field Portable GC-MS (Viking 573)

Carbaryl

Table 4.18 shows data from sampling and analysis with ultra-pure water using the field-portable GC-MS system. Carbaryl was detected and quantified down to a concentration of 0.1 mg/L, which was the same quantifiable level that was determined with the lab-based GC-MS system for the control. However, the field-portable system was not able to detect the compound below this concentration while the lab-system was able to detect carbaryl at a concentration of 0.05 mg/L. Greater variance of peak area occurred within data sets using the field-portable system. Figure 4.9 shows the linearity coefficients ($R^2 = 0.999197$, $R = 0.999599$) that were stronger for the field-system than those seen with the lab-based system. This indicates a very strong correlation between sample concentrations and peak areas. While both systems were able to detect and quantify carbaryl down to 0.1 mg/L, the analysis time using the field-portable system with the resistively heated column was significantly shorter than the lab based system. The analytical method's equilibration and extraction times totaled 25 minutes. In order to meet the objective of detection and quantification of this pesticide within 30 minutes, the field-portable system with the RVM column would be needed. The total analysis time with the field system is 28.7 minutes (carbaryl retention time of 3.7 minutes) while the lab system required an additional 8.2 minutes (retention time of 11.9 minutes). Figures 4.10 and 4.11 show GC-MS chromatograms for both systems. The chromatography peaks for each compound were not as sharp with the field-based system indicating poorer resolution. Each peak showed good separation. In order to avoid the coelution of compounds into one peak, the base peak widths should be minimized when possible to ensure the ability to detect unknowns that tend to elute near the same time as the target analyte.

Table 4.18 – Sensitivity of Fiber and Method for Carbaryl in Control with Field Analytical System
70 µm CW/DVB Fiber, 40°C, and 15 Minute Immersion Extraction

	Control Serial Dilutions (mg/L)					
Sample #	0.01	0.05	0.1	0.5	1.0	5.0
1	NDP	NDP	371,366	840,029	2,933,446	6,932,542
2	NDP	NDP	255,846	1,216,086	1,616,680	11,791,222
3	NDP	NDP	164,731	1,005,641	1,350,628	8,163,227
Mean	0	0	263,981	1,020,585	1,966,918	8,962,330
STD DEV	0	0	103,557	188,473	847,542	2,525,988
RSD (%)	0	0	39.23	18.47	43.09	28.18

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.9 – Detection of Carbaryl in Control with Field Analytical System and 70 µm CW/DVB Fiber

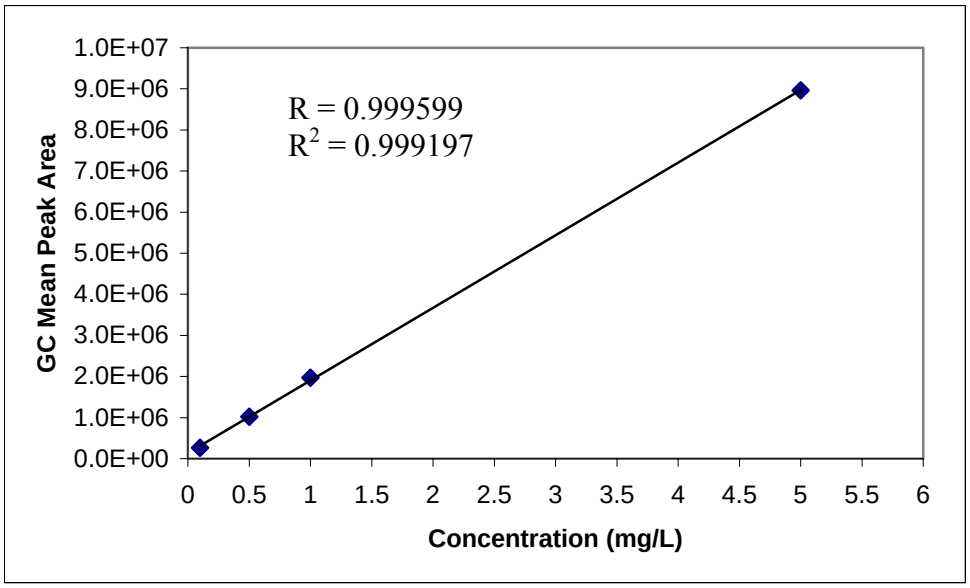


Figure 4.10 – GC-MS Chromatogram for Carbaryl (5.0 mg/L) with Agilent 6890

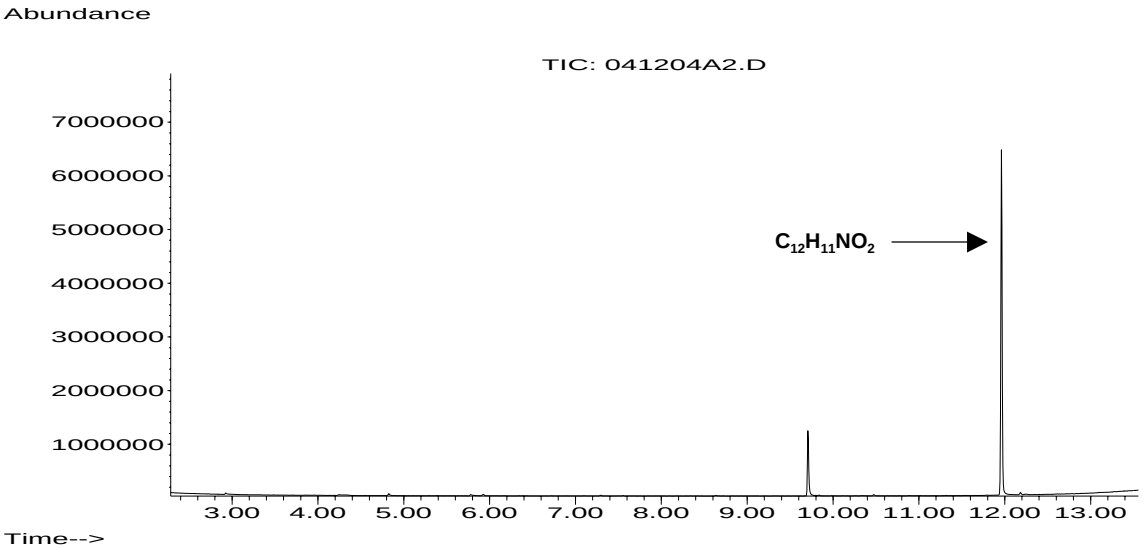
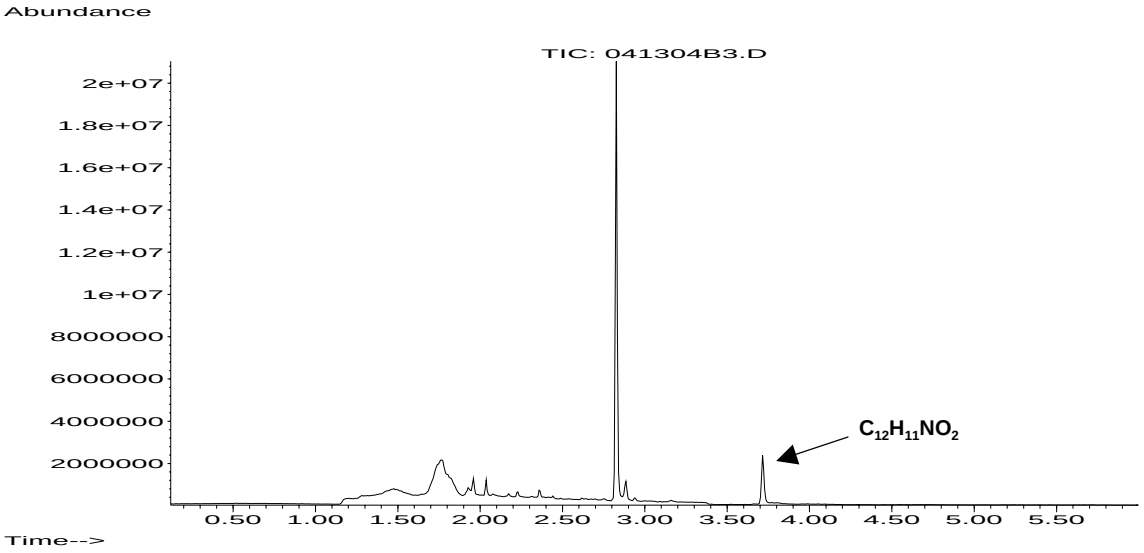


Figure 4.11 – GC-MS Chromatogram for Carbaryl (5.0 mg/L) with Viking 573



Lindane

Table 4.19 shows data from sampling and analysis with ultra-pure water. Lindane was detected and quantified down to a concentration of 0.05 mg/L, which is ten times higher than that seen with the control and the Agilent system. However, peak areas were quantifiable in two of three samples at 0.01 mg/L and in one of three samples at 0.005 mg/L, which was the quantifiable peak for the control with the lab-based system. No peaks were detected below a concentration of 0.005 mg/L. Greater variance within sample sets occurred at the higher concentrations, which is not consistent with other variances observed with this method and system. Figure 4.12 shows good linearity ($R = 0.994464$, $R^2 = 0.988958$) indicating a strong correlation between concentration and peak areas despite the high variance in the one set. The average retention time for the field-system with the RVM column was 3.3 minutes versus 11.3 minutes with the Agilent 6890. Again, as with carbaryl, the total time for analysis with the Viking was 28.3 minutes while it was 9 minutes longer with the Agilent system. Therefore, the 30 minute detection and quantification objective can be met using the field-portable system. Figures 4.13 and 4.14 provide representative chromatograms for the two systems. Similar results and conclusions were apparent for lindane as were observed with carbaryl for the two systems.

Table 4.19 – Sensitivity of Fiber and Method for Lindane in Control with Field Analytical System 30 µm PDMS Fiber, 40°C, and 15 Minute Immersion Extraction

	Control Serial Dilutions (mg/L)							
Sample #	0.001	0.005	0.01	0.05	0.1	0.5	1.0	5.0
1	NDP	77,546	37,414	155,971	408,339	2,651,154	42,575,665	101,717,426
2	NDP	PD/NQ	37,549	124,994	400,773	2,621,452	34,319,801	110,039,364
3	NDP	PD/NQ	PD/NQ	160,047	394,133	2,632,367	11,057,296	132,347,714
Mean	0	0	0	147,004	401,082	2,634,991	29,317,587	114,701,501
STD DEV	0	0	0	19,170	7,108	15,024	16,343,760	15,838,412
RSD (%)	0	0	0	13.04	1.77	0.57	55.75	13.81

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean x 100
NDP = No Detectable Peak
PD/NQ = Peak Detected/Not Quantifiable

Figure 4.12 – Detection of Lindane in Control with Field Analytical System and 30 µm PDMS Fiber

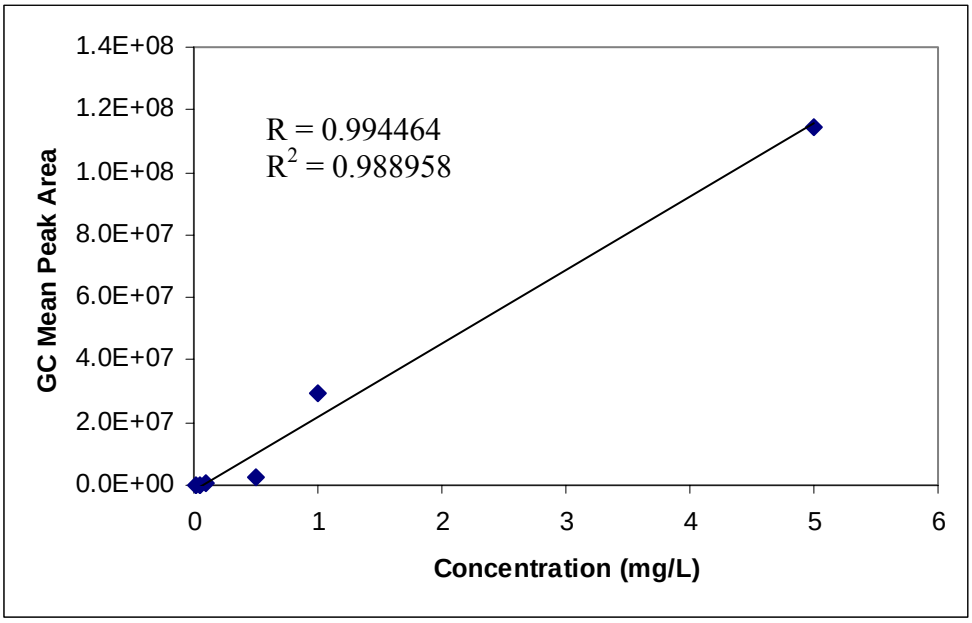


Figure 4.13 – GC-MS Chromatogram for Lindane (5.0 mg/L) with Agilent 6890

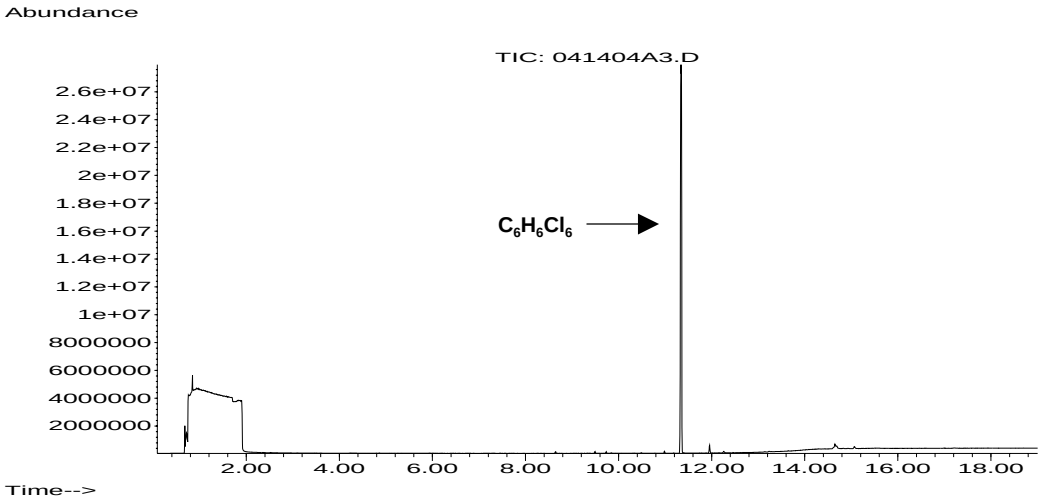
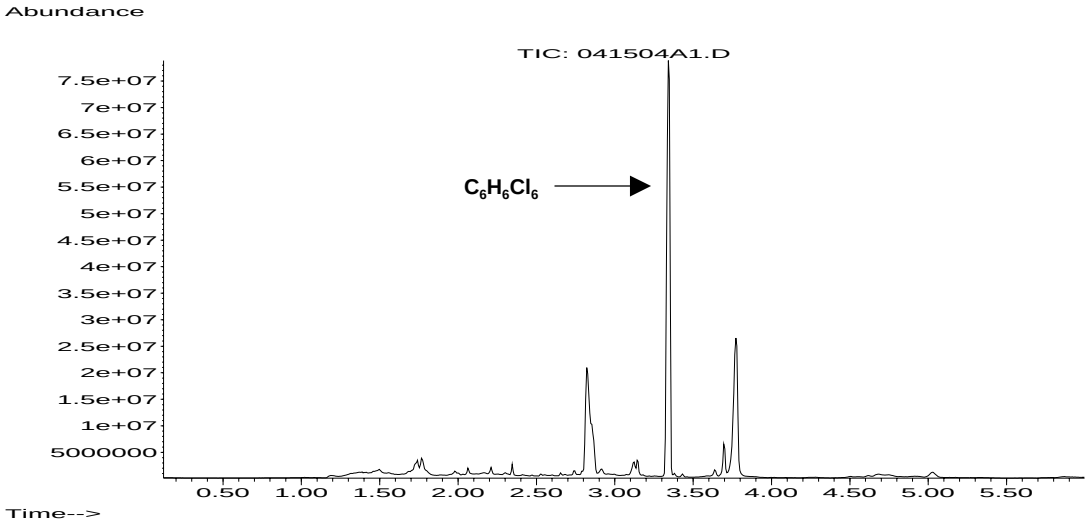


Figure 4.14 – GC-MS Chromatogram for Lindane (5.0 mg/L) with Viking 573



CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Pesticides are extremely valuable compounds used for both agricultural and non-agricultural operations throughout the world. Due to their physical and chemical nature, it can be expected that they will be present in trace quantities in surface and ground water sources. Their presence at low concentrations requires an analytical method that can extract these chemicals from liquid matrices and concentrate them so that detection and quantification is possible. Solid-phase microextraction, coupled to both laboratory-grade and field-portable GC-MS analytical systems, have shown to be an effective sampling and analysis combination capable of detecting and quantifying different classes of pesticides in various environmental waters at, or below, military exposure guidelines (MEG). This study met all objectives of the U.S. Army Center for Environmental Health Research's science and technical objective. The fibers and method achieved adequate sensitivity since they produced detectable and quantifiable results below the MEGs. The sampling and analysis methods proved to be robust as they were able to detect low concentrations of carbaryl and lindane in a variety of different water sources, including raw surface water. Finally, the field-portable GC-MS system, using the LTM/RHC technology, achieved a total sampling and analysis time of less than 30 minutes, demonstrating near-laboratory quality data is rapidly available in the field.

Quantitative results were achieved in this study since a range of known concentrations was spiked into each water source. However, for analysis in a field environment with GC-MS, the resulting peak area (abundance) of a compound detected in a sample would not be quantifiable unless an internal standard was added to each sample or a calibration curve was created using

analytical standards of known concentration. This would need to be done in water similar to that being sampled in order to account for matrix effects from materials contained in the water.

The comparative results achieved for the two analytical systems indicate that the field-portable GC-MS system, using the LTM/RHC technology, can achieve a level of sensitivity comparable to that achieved by the laboratory system. However, the variation between samples using the field-portable system was higher than that observed with the laboratory system. The use of the computer controlled auto-sampler on the laboratory system allowed for greater precision with the sampling technique between individual samples. In addition, the auto-sampler's built-in hot-block and magnetic stirrer were better suited to control the temperature and stir rate of each sample matrix. The manual extraction techniques used in the field-environment most likely accounted for the higher variance observed across each set of sample concentrations while using the field-portable system. Furthermore, the sample temperature was inconsistent across individual samples using the heater on the magnetic stir plate since the temperature was not precisely controlled with computer software and the vials were heated only on the bottom while the sides of the vials were exposed to the ambient air during the equilibration and extraction periods.

This work demonstrated that SPME with GC-MS analysis could be used as a quick screening method of water sources and supplies. This can be an effective analytical tool for use by military forces to quickly screen environmental water sources that have been identified as potential source waters for use in the production of drinking water supplies. It can also be used for periodic analysis of post-production supplies to ensure they have not been contaminated with pesticides. This system can also be an effective tool for public health and regulatory agencies to conduct on-site field analysis of water sources used for municipal drinking and recreational

water supplies, for wastewater effluents, and for environmental compliance and enforcement. SPME sampling, coupled to GC-MS analysis using field-portable systems, can provide rapid results from environmental water sampling that are needed by both military and civilian health and safety planners to make quick and informed decisions that help to ensure the necessary safeguards are in place to protect these vital commodities and the health of deployed military forces and civilian populations.

Recommendations

Currently, there are very few military units in the Department of Defense that possess GC-MS equipment and have personnel who are properly trained to use the equipment. Environmental analysis is not a primary function of these units. However, the rapid detection method developed during this research project has proven to work for the detection of pesticides from two separate classes at concentrations below MEGs. If environmental samples were obtained in a field environment, they could be sent to these units who could implement these methods to quickly detect the presence of pesticides and other chemical contaminants that are harmful to human health. Timely information can be effectively used by military commanders to quickly assess the threat posed to their troops from contaminants found in water so that control measures can be implemented to reduce exposures and safeguard human health.

The LTM/RHC technology is currently a piece of equipment that can be added on to a field-portable GC-MS system. The current placement of these modules on the GC-MS equipment makes them prone to damage since they are located on the exterior of the equipment. This would be especially true if the entire system were mounted in a van for use as a mobile field laboratory. Through the continued advancement of analytical equipment technologies, and innovations in

manufacturing, GC-MS systems continue to get smaller. The LTM/RHC technology has proven to be a beneficial application to field-portable GC-MS systems and should be permanently implemented into the design of these systems to make a system capable of withstanding the harsh conditions encountered in field environments.

The methods developed in this study have shown to provide sensitive and precise detection capabilities for pesticides found at trace levels in various environmental water sources. These methods can be used by public health agencies at all organizational levels to augment current field analytical equipment to rapidly detect the presence of pesticides and other chemical contaminants in ground and surface water resources and in post-production water supplies. Quantification of pesticides in water sources and supplies is possible with the use of pure analytical standards to produce calibration curves that would allow for the extrapolation of the concentration of the compounds found in a liquid matrix. These methods can also be used in water and wastewater treatment facilities that use biological treatment systems for water purification. Biological treatment systems are susceptible to chemical contaminants and require periodic monitoring for the presence of chemical contaminants. The equipment and methods used in this study would be very well suited for the detection of chemical contaminants in such treatment systems.

Limitations of Study

One limitation of this study was the use of multiple stock solutions. Smaller stocks were made since a large refrigeration system was not available to store one large stock solution for each pesticide. The use of one stock solution for the entire study would be ideal to ensure better

statistical reliability and comparability between data sets. However, if one stock solution was used throughout the entire study, the potential for compound degradation is likely during an extended storage period. Degradation was mostly a concern for carbaryl and could have resulted in findings that were not comparable in later stages of the study. However, since analytical results were not compared between the different phases of the study, it was not essential to use the same stock solution throughout the study. Therefore, smaller stock solutions were prepared just prior to the start of each phase, used for an entire phase, and stored for no more than 14 days. Using a single stock solution for each phase helped to reduce the potential for compound degradation and ensured the results were consistent and comparable for subsequent trials within each experimental phase. Also, since all stocks were prepared in the exact same manner and under the same conditions, it was expected that the results produced from different stock solutions would be similar.

A possible second limitation of this study was the use of immersion SPME over headspace SPME. The techniques used in headspace sampling are slightly different than those used in immersion sampling. Different adjustments like altering the pH of the solution and optimizing the stir rate would have been important to drive an anaylte into a sample's headspace for extraction. However, these parameters were not optimized for immersion extractions. The pH of all water sources used in this study ranged from 4.5 to 7.0. Other research concluded that altering the pH of a solution did not change the results and was not required prior to SPME sampling[27,28]. Therefore, it was not necessary to optimize this parameter. The scientific literature indicates that the rate of stirring can affect how an analyte is distributed in solution and affect its transport into a sample's headspace. Stirring also helped to reduce the effect of a "depletion zone" that is produced around a fiber as a result of slow diffusion transport of an

analyte through the stationary layer of liquid surrounding the fiber[14,15]. Other research conducted for the extraction of lindane from water concluded that the recovery percent for immersion extraction was over twice as high as that observed from headspace sampling[31]. Since the pesticides under investigation have high molecular weights and are not very volatile, immersion sampling is the most appropriate SPME method for this study. Also, since stirring can improve recovery rates for immersion sampling, a stir rate should be used that is just lower than the rate that produces a vortex in the sample. The highest rate of stirring allowed by the auto-sampler's software did not produce a vortex and was used consistently throughout the study. It is not expected that the results would differ significantly if a higher stir rate were used. For these reasons, immersion sampling was selected as the appropriate extraction method for this study.

A third limitation of this study is the use of only four water sources. Differences in water composition and chemistry can vary across and within geographical regions, which could have an impact on the extraction, detection, and quantification of the pesticides. For example, the modeled ground water may not be representative of the many different compositions of ground water potentially available for environmental samples. Secondly, calcium hypochlorite was added to the ultra-pure water to produce chlorine residual to simulate post-production water. Calcium hypochlorite is an acceptable compound for this purpose, but the residual concentration was slightly higher than is used for most public water systems, which is usually only present in trace quantities (< 1.0 ppm). Therefore, the results achieved in this work may not be directly applicable to all water sources.

A final limitation of the study may have been with the analytical instrument chosen for the study. Gas chromatography is a widely accepted analytical instrument for the analysis of toxic industrial chemicals and is appropriate for volatile and semi-volatile chemicals. A mass spectral

(MS) detector was used in full scan mode, which does not produce the same level of sensitivity that can be achieved with an electron capture detector (ECD). The sensitivity of the method may have been improved through the use of an ECD; however, this would significantly reduce the ability to identify unknown chemicals in the sample. An MS detector was required for this study since it was important that unknowns could be detected in a mixture. The MS detector provides an additional advantage since it can be used in a selected-ion mode. If a compound is detected in a sample using the full scan mode, the compound can be targeted in subsequent samples using single-ion mode, which produces better sensitivity than the ECD. This ability makes the MS detector best suited for both detection and quantification of pesticides in the field.

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